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THE CHEMICAL PROPERTIES OF *DERRIS ELLIPTICA* (TUBA ROOT)

BY F. TATTERSFIELD AND W. A. ROACH.

(*Rothamsted Experimental Station.*)

(With 1 Text-figure and Diagram I.)

HISTORICAL.

TUBA root first interested Europeans as being a constituent of the "ipoh" arrow poison of the Malays(1) and of the "siren" arrow poison of Borneo(2). For this purpose the green root was macerated with water, the arrow head alternately painted with the resulting milky fluid and dried in the sun until a sufficiently thick coating of poison was formed.

Latterly its chief use has been as a fish poison; though at present such use is prohibited. It is now employed by Chinese market gardeners as an insecticide and there is a growing demand for it on the English market.

BOTANICAL.

Tuba root (*Derris* (*Pongamia*) *elliptica* Benth.) belongs to the papilionaceous legumes, which include many intensely poisonous plants such as Pongamia, Millettia and Tephrosia, all nearly allied to Derris. A botanical description of the many closely related species of this plant is given in Brandis' *Indian Trees*. As early as 1848 Oxley(3) used a decoction of the root as a means of controlling pests of the nutmeg tree. Attention was drawn to the root again in the *Kew Gardens Report* for 1877(4) where it was suggested as an insecticide. In 1886 it was exhibited in the Straits Settlements Courts of the Colonial and Indian Exhibition at South Kensington. The desirability of chemical and physiological examination and its possible use as an insecticide were pointed out in the *Pharmaceutical Journal*(5). Wray, in 1882, stated that the Chinese market gardeners were using it for this purpose. The root was chopped up fine in the fresh state, then pounded and mixed with water. The resulting milky liquid was sprayed or brushed over the plants with a bunch of feathers.

The report in the *Pharmaceutical Journal* of the Colonial and Indian Exhibition, referred to above, led Greshoff(6) to undertake a chemical examination of the root. He extracted the coarsely-ground root bark with water in a percolator to free it of tannins and mucilage. After drying, it was extracted with alcohol; to the extract water was added to precipitate the resinous poisonous principle. The alcohol was evaporated off, leaving the "derrid" in a semi-fluid state under a milky supernatant fluid. This crude derrid was purified by treatment with dilute alcohol, followed by 5 per cent. alkali; it was then dissolved in chloroform, washed with water, the solution digested with animal charcoal, and concentrated, whereupon yellow acicular crystals separated out. The purified derrid separated out as a resin on evaporating off the chloroform. It could be fractionated by dissolving in pyridine and adding petroleum ether gradually, the most highly coloured portions separating out first. Greshoff however failed to isolate any white crystalline product. He points out that the poisonous principle is neither a glucoside nor an alkaloid. He describes it as a resinous body of acid reaction, M.P. 61° C. (crude substance) which decomposes at 160° C. giving off an odour resembling coumarin. On heating with caustic alkali it yielded salicylic and protocatechuic acids. He also found that one five-millionth part of the resin stupified goldfishes in a few minutes and killed them in less than half an hour. Thus it is one of the most violent fish poisons known.

Wray junior(7) separated the toxic constituents by warming the chopped and crushed root with alcohol acidified with hydrochloric acid for some hours, filtering and evaporating on the water bath at a low temperature until a gummy substance separated. This he named "tubain." When all the alcohol was evaporated, the tubain was removed and washed by kneading in hot water and further purified by re-solution in alcohol and repeating the above process. A dirty white crystalline substance separated out from an alcoholic solution of tubain on standing. This substance, he states, was not poisonous to fish when freed from tubain. The poisonous principle was not an alkaloid. It gave a dragon's blood red solution with nitric acid. Wray found that ·03 per cent. of the green root in water sufficed to kill fish. Some of the physiological effects on fish are mentioned in the paper. ·00029 per cent. of the resin was quickly fatal to fish, while ·00001 per cent. killed in from 15 to 30 minutes, according to the species. From the fact that fish may eat fairly large amounts with impunity, he argues that the insolubility of the substance in water, renders it necessary to use it in the form of an emulsion, in which form doubtless it exists in the milky sap of the plant. He suggests

that we follow the lead of the Chinese gardeners and use it as an insecticide. For this purpose Wray points out the dried root would be of little or no use. The poison would have to be extracted and converted into an emulsion or into some chemical combination easily dissolved in water.

In 1889 Sillevoldt(8) published the results of his chemical investigations. He extracted the root with water, dried, then extracted with boiling 96 per cent. alcohol. The resinous product was dissolved in ether and precipitated with petroleum ether. He states that if the resin be boiled with alcoholic hydrochloric acid the anhydroderivative $C_{33}H_{28}O_9$, $\frac{1}{2}H_2O$ is obtained. This is contained in the original resin "derrid" which has a formula $C_{33}H_{30}O_{10}$ when pure. The anhydroderivative crystallises in fine light yellow needles, M.P. 214° C. Both the above compounds contained three (OCH_3) groups. By treating these with hydriodic acid a body of constitution $C_{30}H_{19}O_6(OH)_3$ was obtained, M.P. 240° C. He was unable to prepare the acetyl or benzoyl derivatives. Sillevoldt apparently failed to isolate the white crystalline product, or none was present in the sample on which he worked. The anhydroderride was not found poisonous to fish.

Power(9) dealing with the stem of a closely allied plant, *Derris uliginosa*, makes some interesting observations in regard to its poisonous action towards fish.

In 1916 Campbell(10) published work from the physiological standpoint. An interesting point in his paper is that the root acts as a stomach poison towards monkeys.

Ishikawa(11) investigated a white crystalline product, isolated by him from the root, which he named "tubatoxin," though the name toxin is hardly warranted by his observations, as the compound is not a protein and there appears to be no evidence of it having the property of inducing the formation of an antitoxin after administration to an animal. (The original paper however was not accessible to us.) The crystals vary in shape but are mostly hexagonal or acicular, soluble in various organic solvents, M.P. 163·3° C., formula $C_{18}H_{18}O_5$, not hygroscopic, burn with a peculiar odour, when heated on platinum foil, leaving no ash, reduce ammoniacal silver nitrate and alkaline copper sulphate, not affected by cold concentrated alkalis, but destroyed by concentrated sulphuric acid.

He gives the following test for the substance. The solution in glacial acetic acid gives a yellow colour on the addition of one or two drops of fuming nitric acid, followed by dilution with water. When the resulting solution is added to dilute sodium hydrate a ring appears in the line

of contact coloured green above and reddish brown below. This reaction reveals the presence of ·001 gm. of tubatoxin. We find that a similar colour reaction is given by the resins, although the actual shade is not identical with that shown by tubatoxin. The symptoms produced in animals (fish, rat, frog, rabbit and dog) were general motor paralysis with dyspnoea of central origin. Blood pressure was first raised, then markedly depressed and palpitation of the heart gradually ceased. When injected intravenously the minimum lethal dose for the rabbit was ·0009 gm. per kilogram of body weight. On subcutaneous injection, tubatoxin was absorbed with difficulty.

The most recent paper on the subject is that of McIndoo, Sievers and Abbott(12) who treated it from the entomological and practical sides only. Their conclusions were:

1. *Derris elliptica* and *Derris uliginosa* act both as contact insecticides and as stomach poisons, affecting different classes of insects according to the development of their nervous systems.

2. Denatured alcohol was found to be a good economic solvent for extracting the toxic principle, which, when applied in spray mixtures, proved to be efficient against certain aphids, potato-beetle larvae, and small fall webworms.

3. Derris used as a dust under practical conditions was found to be efficient against dog fleas, chicken lice, house flies, three species of aphids (*Aphis rumicis* L., *A. pomi* De Geer, and *Myzus pessicae* Sulz.), potato-beetle larvae, and small fall webworms, but of no practical value against bedbugs, roaches, chicken mites, mealybugs, orthezia insignis, red spiders, or against the crawling young of the oyster shell scale. Used as powder in water with or without soap under practical conditions, it proved to be efficacious against most of the aphids sprayed, and also against cabbage worms (*Autographa brassicae* Riley), the larvae of apple datuns (*Datana ministra* Dru.), oak worms (*Anisota senatoria* S. and A.), small tent caterpillars and potato-beetle larvae.

After the work for this paper had been completed, we were favoured with notes on investigations carried out between 1902 and 1907 by Dr H. E. Durham; for commercial reasons his results have not been published. In general, Durham's conclusions in regard to the insecticidal properties of Derris are concordant with ours; thus larvae of lepidoptera and sawflies are very susceptible, and aphides (*Bean aphis, A. rumicis* and *Woolly aphis, E. lanigera*) quite resistant to wet application. Mosquito larvae and pupae, fresh water crustaceans and molluscs, also tadpoles and fish all formed usefully susceptible test animals. Durham

insists on the existence of the active principles in a crystalline and a resinous form (cf. van Sillevoldt), and states the former is best obtained from the original petrol ether extract freed from resin first by cold absolute alcohol and then repeated crystallisations from the same solvent heated, until the laminar acicular crystals of constant M.P. at 164½° C. are obtained. Both of these active principles give (*pace* the impossibility of getting a crystal-free resin) a characteristic and intense red colour reaction with strong nitric acid which contact with a drop of ammonia changes to an evanescent deep peacock blue-green. Tests for nitrogen, sulphur, glucosides and cholesterols (*v.* Power) proved negative. Owing to lapse of time and loss of some notes further details of other chemical and biological trials (frogs' hearts etc.) cannot be given here. As regards insects he concludes that toxic effects are due to absorption from the alimentary canal rather than from surface contact.

EXPERIMENTAL PART

Separation and Description of Constituents.

The root was extracted with 95 per cent. alcohol. Owing to the chemical change undergone by tubatoxin on boiling with this solvent, it was found necessary to carry out the process at a temperature not exceeding 30° C. Large amounts of the powdered root had to be extracted, and as the usual methods at our disposal entailed considerable labour and large losses of solvent, a suitable automatic percolator was devised, of which Fig. 1 is a diagram.

The container, *A*, into which the powdered root is placed, is an inverted bell-jar. Another similar jar, *B*, is placed over *A*, an annular rubber ring making an airtight joint between them. It is a convenience to have a tubulure and tap *D* in *B*, but a T-piece and a tap between *C* and *B*, would be effective. A two-litre round-bottomed flask *F*, the neck of which has been cut off short, is connected with the bottom of *A* by tube *E*, and with top of condenser *C* by tube *J*, which should have a sufficiently wide bore to allow any condensed alcohol to flow freely back into the flask *F*. Bumping is prevented by the introduction of a capillary *P*. Tube *H* and funnel *G* are used for the withdrawal and introduction of liquid. *F* is partially immersed in a water bath, kept at a constant temperature of 30° C. by means of a thermo-regulator. To fill the apparatus it is disconnected at *L*, and *B* and *C* taken off together. The rubber ring is removed. A suitable filter is placed in the neck of *A*; if the material is coarse, a plug of glass wool is sufficient, if fine as in our

case, teased-out cotton wool must be used. The rate of percolation can be increased through the enlargement of the filtering surface, by placing sand above the wool to about *M*. The substance to be extracted is placed in *A*. The top rim of *A*, the bottom one of *B*, and the rubber ring are thoroughly cleaned, and the apparatus set up as shown in Fig. 1, connection made at *L* and the whole apparatus evacuated through *D* to

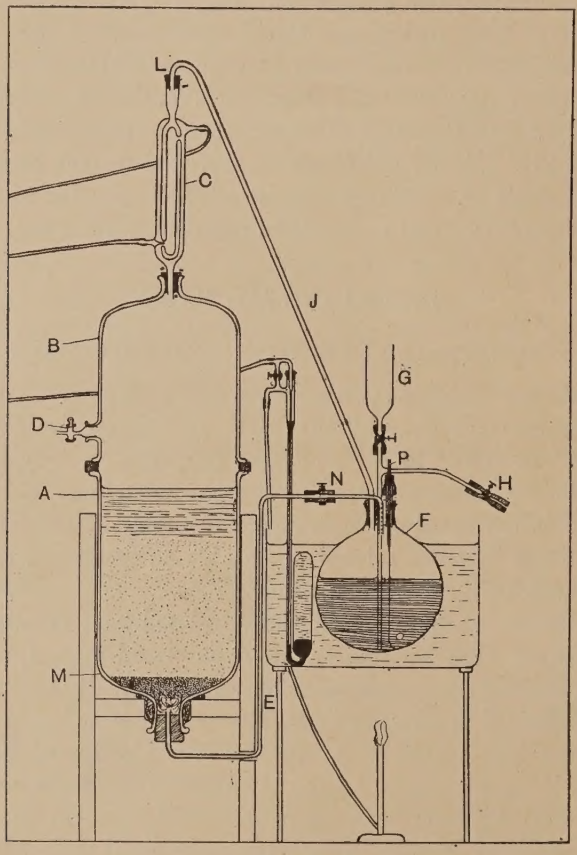

Fig. 1.

get rid of the air enclosed in the capillaries of the powder. When the apparatus is evacuated, alcohol is introduced through *D*, by suction. After the alcohol has percolated through the powder it is necessary to complete the evacuation of the apparatus. To preserve a good vacuum in the apparatus, it may be necessary to smear the outside of the rubber ring with vaseline or other thick grease. There is practically no danger

of the vaseline being dissolved by the solvent if put on after the apparatus has been set up. The filtered alcoholic extract passes via tube *E* to the flask *F*. The alcohol boils under the partial vacuum in *F* and the vapour, after passing up the tube *J*, condenses in *C* and drips into *A*. In our procedure when the extract became concentrated in *F*, fine crystals separated out. These were removed by evacuating through a filtering flask at *H*, the crystals filtered off and the extract returned through the funnel *G*. On completion of the extraction the highly concentrated extract can be removed by suction through *H*, after which the excess of alcohol in *A*, can be syphoned off into *F* and through *H* by suction, the process being much speeded up by removing *B*, closing tube *J* and placing weights upon the extracted powder in *A*. The last traces held up by the powder, were removed by transferring to a steam jacketed vacuum pan, to which was attached a condenser. By adopting this practice very little alcohol need be lost. The apparatus with us proved very convenient; it may require evacuating occasionally, but otherwise it runs automatically.

The highly concentrated extract is then filtered and the mass of impure crystals, and deep brown solution so obtained, treated separately. The crystalline product is best purified by drying in the steam oven, after being filtered free from alcohol. This drying seems to render insoluble some of the dark viscous material with which the crystalline mass is contaminated. In cases where the original product was very impure, we found that the best procedure to adopt was to dry the crystals from benzene crystallisation, moisten with alcohol, re-dry in the oven and then proceed with the recrystallisation from benzene. This last procedure however was only found to be necessary when from any cause the extraction apparatus was allowed to stand a long time after the extraction was complete, in which case the crystals were cemented by resinous matter. In this way we were able to isolate "tubatoxin," a white crystalline product previously obtained by Ishikawa (M.P. 163° C.) and two yellow crystalline products, one of which corresponded to the anhydroderride of Sillevoldt, M.P. 214° C. Both these products are distinctly less soluble than "tubatoxin." Care and speed have to be exercised in the recrystallisation, particularly if alcohol be used, as the "tubatoxin" readily undergoes change, a dirty brown product being the result. This product on fractional crystallisation was resolved into a resin (which can be fractionated to some extent by precipitating its ether solution by pentane or petroleum ether), and three yellow crystalline products. Two of the latter correspond to the two isolated from the extract of the root, while the other had a melting point of

222–224° C. It seems probable therefore that the yellow crystals isolated by us, and by Sillevoldt directly from the root extract are products of chemical change undergone by "tubatoxin" during the process of extraction. Sillevoldt describes the preparation of anhydroderride from the resins isolated from the root, by boiling them with alcoholic hydrochloric acid; but we were not able to confirm this, provided that pure resin free from "tubatoxin" was used; but the change undergone by "tubatoxin" on boiling with alcohol goes smoothly and easily. The supernatant liquid filtered from the crystalline products was treated with basic lead acetate, only the quantity necessary to precipitate tannins, gums etc., being used, as it was found that the resins themselves are brought down by an excess, and their recovery was a matter of some difficulty; we had evidence also that the product derived from the lead acetate precipitate of the resins, was different from the original resins. The lead precipitate was separated and the filtrate afterwards aerated to free from sulphuretted hydrogen, the alcohol distilled off *in vacuo*, taken up with ether, filtered from any crystals, and precipitated by pentane in fractions. The final fraction soluble in ether-pentane, evaporated at ordinary temperature, was thoroughly extracted with pentane to free from "tubatoxin." This solution on evaporation gave a small quantity of liquid resin which could be partially purified from "tubatoxin" by taking up with a small quantity of pentane, cooling in a solution of solid CO_2 in ether, and filtering while being cooled in the same medium. The oil was never freed entirely from "tubatoxin," as we had only a few drops on which to work at any one time. The resins which were golden yellow in colour had the following melting points.

Fractions:	(1) 70–90° C.	(3) 62–76° C.
	(2) 72–82° C.	(4) 60–64° C.

All these resins and the oil proved toxic to insects, the oil however probably owed its toxic properties, at any rate to some extent, to the small amount of "tubatoxin" from which we were never able to free it. The dark brown lead precipitate was worked up by suspending in alcohol, saturating with H_2S, filtering and after aeration evaporating to dryness. A brown amorphous product resulted. It was non-toxic to insects. An ultimate analysis of "tubatoxin" and the resins, showed only the presence of C, H and O. The most characteristic feature of the crystalline products and the resins, was the presence of methoxyl groups. The fact that "tubatoxin" is readily converted into a resinous substance, partially precipitated from ether solution by pentane and of a melting point 62–72° C.

and into crystalline yellow derivatives indicates that all these products are inter-related and probably have a common derivation. In a schematic form the process is expressed in Diagram No. 1, in which toxic constituents are indicated by means of heavy lines.

SEPARATION OF CONSTITUENTS OF TUBA ROOT.

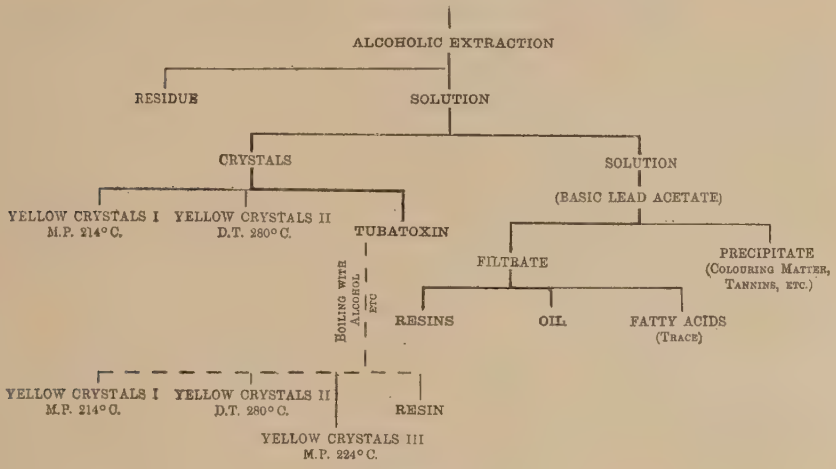

Diagram 1.

PROPERTIES OF THE CONSTITUENTS OF THE ROOT.

"*Tubatoxin.*"

(1) Colourless crystals.

(2) Soluble in most organic solvents, insoluble in water.

(3) Forms molecular compounds with various organic solvents, notably benzene and alcohol.

(4) Its ether solution not precipitated by pentane.

(5) Contains only carbon, oxygen and hydrogen.

(6) Heated to 100° C. in a current of oxygen or carbon dioxide resinifies, becomes brown in colour. The rate of change is slower in carbon dioxide.

(7) Contains 15·4–15·6 per cent. CH_3O.

(8) Alcoholic solution reduces ammoniacal silver nitrate.

(9) On boiling with alcoholic caustic potash, turns deep red in colour, and gives rise to a saponifiable and an unsaponifiable portion—both are resinous.

(10) On boiling with alcohol (95 per cent.) gives rise to a series of

yellow crystalline derivatives and to a resin melting at 62–72° C. A trace of moisture accelerates this change.

(11) Undergoes chemical change when its solutions are exposed to sunlight or ultraviolet light.

(12) Is toxic to insects.

Nos. (3), (6), (10) and (11) are considered in detail below.

(3) *Solvent of crystallisation of "Tubatoxin."* Small samples of crystals from benzene were warmed in a sprengel pump vacuum, and crystals from benzene and alcohol dried in the steam oven with the following results. 129·3[1] parts of crystalline product from benzene gave 100 parts pure "tubatoxin" on drying three weeks in Sprengel vacuum. 119 parts of crystalline product from benzene gave 100 parts pure "tubatoxin" on drying one week in steam oven. 103 parts of crystalline product from alcohol (needles) gave 100 parts pure "tubatoxin" on drying to constant weight in steam oven. 100 parts of crystalline product from alcohol (plates) gave 100 parts pure "tubatoxin" on drying to constant weight in steam oven.

From the Sprengel pump vacuum test we were able to collect a little clear liquid which had a boiling point of 80° C. and melting point of 4–5° C. and answered the tests of benzene.

These experiments show that we have definite proof of one crystalline product with benzene of crystallisation and of one with alcohol (needles). The plate crystals from alcohol appear to be free from solvent of crystallisation.

(6) *Effects of heat on the toxic constituents of the Root.* "Tubatoxin," resins and oil were weighed out into porcelain boats, and kept at 100° C. for some days in an atmosphere of oxygen and a control set kept at the same temperature in carbon dioxide.

The loss was less in each case in oxygen than in carbon dioxide. "Tubatoxin" in CO_2 gave rise to a product coloured purplish-brown. "Tubatoxin" in oxygen gave rise to a product coloured brown. Under the microscope the latter showed crystals changed in places to a yellow product. The resins appear to suffer no loss of solubility by heating in carbon-dioxide but suffered very remarkably in oxygen. There was much greater darkening in colour in oxygen than in carbon dioxide. The oil darkened in colour in each case but also resinified in oxygen.

(10) *The change of "Tubatoxin" on boiling with alcohol.* This has already been briefly considered; it has not been completely worked out, but is full of interest. Traces of moisture accelerate it. "Tubatoxin" solu-

[1] Owing to an accident it was impossible to continue this to constant weight

tions in absolute alcohol do not change in colour on boiling so readily as do those prepared with 95 per cent. alcohol. Equal quantities therefore of the same colourless solution of "tubatoxin" in alcohol were poured into two tubes. To one was added a few drops of water and they were heated on a water bath. After half an hour the tube containing water was markedly more yellow than the other, which remained practically colourless.

(11) *Colour Change of "Tubatoxin."* We have already seen that on exposure to light an alcoholic solution of "tubatoxin," or more so on boiling, gives rise to a yellow solution (p. 9). It was observed that dry crystals of the substance stored in a tube became a pronounced yellow on the side of the tube more exposed to the light, without suffering any appreciable change of colour in sheltered parts of the tube. As it was necessary to carry out insecticidal tests with this material in as pure a condition as possible it was considered desirable to ascertain how rapidly this colour change could be induced. A 4 per cent. solution in carbon tetrachloride which was colourless when made up became a reddish-yellow on standing in the light for some weeks. A slight reddish-brown precipitate was also formed. This solution was filtered and 20 c.c. introduced into each of the receptacles of the Duboscq colorimeter. No. 1 was kept in the instrument where it was screened from any direct light, and No. 2 was exposed to the light from a mercury lamp. After two hours and four hours respectively, their relative colour intensities were measured, with the following results:

	Depth of liquid in tube		Intensity of colour 1 / Intensity of colour 2	Mean
	1	2		
After 2 hours	3·0	2·83	·94	·85
	2·5	2·07	·83	
	2·72	2·59	·92	
	3·10	2·44	·80	
	3·10	2·72	·88	
After 4 hours	2·27	1·60	·70	·67
	2·0	1·38	·69	
	2·0	1·23	·62	

Hence there was a marked increase in colour on exposure to the light from a mercury lamp.

Resins.

1. Yellow to light brown in colour.

2. Precipitated by an excess of basic lead acetate to give a light brown product.

3. Readily soluble in most organic solvents, with the exception of pentane, petroleum ether and water.

4. Partially precipitated from methylated ether by pentane and petroleum ether. It can be partially fractionated in this way. The following fractions were thus obtained by us:

A 1. Dark coloured, very small quantity, M.P. 70–90° C.

A 2. Light yellow, M.P. 72–82° C. methoxyl content 16·0 per cent.

A 3. Light yellow, M.P. 62–76° C. methoxyl content 15·3 per cent.

A 4. Soluble in pentane ether. Insoluble in pentane, M.P. 60–64° C. methoxyl content 13·8 per cent.

5. Poisonous to insects.

Liquid resin.

1. Light yellow coloured oil, soluble in pentane.

2. Partially resinifies on standing.

3. When purified as far as possible, has a methoxyl content of 2·85 per cent.; this was probably due to presence of traces of tubatoxin, as on progressive purification the methoxyl value decreased in amount from 4·6 to 2·85 per cent.

4. Darkens in colour and resinifies on heating at 100° C. in oxygen.

Yellow Crystalline Derivatives.

1. Three of these were separated having melting points of 213–214° C. 224–226° C. while a third set of crystals decompose at 280° C. without melting.

2. Crystallise in long needles.

3. They are all only slightly soluble in organic solvents.

4. Methoxyl content 16·6–16·8 per cent.

5. Do not precipitate silver from alcoholic ammoniacal silver nitrate.

There was a wide variation both in the total amounts, and the relative proportions of the various constituents of the roots. Some consignments contained as much as 20 per cent. of poisons while in others it fell as low as about 5 per cent. The non-poisonous constituents also varied considerably. Moreover in some of the roots the amount of tubatoxin present was not great while in others it formed one-third of the toxic constituents.

The approximate yields of the various constituents not precipitated by basic lead acetate in the poorest quality of root submitted to us are given in Table I.

In the second experiment the resins were slightly contaminated and the amounts of pure resin were calculated from their methoxyl contents.

Taking the figures in the first set and the amount of each constituent and its CH_3O value, the calculated value for the CH_3O content for the whole extract of poisons is 14·6 per cent.

An extraction with carbon tetrachloride gave 4·73 per cent. of extract with a methoxyl content of 14·7 per cent.

Table I.

Constituent	Experiment 1		Experiment 2	
	% on root	% CH_3O in constituent	% of impure constituent on root	% pure constituent on root
Tubatoxin	1·48	15·65	1·33	1·33
Resin precipitated from ether solution by petroleum ether 2 : 1	1·29	15·79	1·78	1·37
Resin soluble in ether and petroleum ether 2 : 1	1·39	13·94	1·23	1·09
Liquid resin	0·22	4·6	0·29	0·29
	4·38		4·63	4·08

THE INVESTIGATION OF A METHOD OF EVALUATING THE ROOT.

The results obtained by the approximate analysis of the root and the investigation of the various constituents indicate a ready method for evaluating the root by chemical means. This is very necessary as the different samples tested by us were found to vary widely in their toxicity to insects and the methods of quantitatively testing them for their insecticidal properties is both difficult and tedious, and have only been partially investigated and moreover are likely to give results of differing value according to the type of insect chosen, the number available, the season of the year and the method of application. Three different consignments of root were examined, they are labelled for convenience *X*, *Y* and *Z*.

Derivation of *X*. Penang.
„ „ *Y*. Kuala Lumpur.
„ „ *Z*. Kuala Lumpur.

They were carefully sampled and the soil detached by picking over, and ground to an almost impalpable powder.

Moisture content. As the products of the powdered root undergo chemical changes on warming, it was found necessary to carry out this estimation at the temperature of boiling dichlorethylene, B. Pt. 55–58° C.

in partial vacuum, over P_2O_5. Even at this low temperature some slight chemical change took place.

Results:	Root *X*.	(1) 8·64	(2) 8·61
	„ *Y*.	(1) 8·50	(2) 8·54
	„ *Z*.	(1) 9·65	(2) 9·49.

Five grams of the finely divided root were weighed into glass vacuum drying bulbs containing glass stoppers fitted with sealed-in glass tubes into which light plugs of cotton wool were placed, one tube being closed by a rubber tube and glass stopper the other being connected through two flasks in series containing P_2O_5 to the water pump. They were kept thoroughly evacuated and dried till the weight reached a minimum.

Conditions necessary for the Estimation of Toxic Extract.

This is based upon the extraction of the powdered root by dry, alcohol-free solvents, preferably ether. Difficulties were encountered in working out this apparently simple method, widely varying results being obtained for the quantity of the extract according to the solvent used, the degree of fineness of the root, the amount of moisture present in the root, the temperature of the extraction and the way in which the extract was dried. These variations are due to a number of factors.

(1) The extraction must be selective.

(2) The root is very impermeable to solvents and so must be ground to an almost impalpable powder, moreover if this is not done there are difficulties in weighing out a true representative sample of the root.

(3) The root must be extracted at fairly low temperatures, as otherwise chemical changes take place which may give rise to sparingly soluble compounds.

(4) The extract undergoes chemical change on drying.

(5) The type of extraction flask may modify results.

(6) The toxic constituents are freed from the last traces of solvent with great difficulty, as illustrated by the case of "tubatoxin" (p. 10).

Difficulties 4, 5 and 6 can be illustrated in the case of the carbon tetrachloride extract of a sample of root. Short drying in vacuum desiccator plus $1\frac{1}{2}$ hours at 100° C. in round-bottomed flask. Per cent. extract on dry root = 5·27.

Prolonged drying at 100° C. in round-bottomed flask. Per cent. extract on dry root = 4·92.

Prolonged drying at 100° C. in flat-bottomed flask. Per cent. extract on dry root = 4·51.

Difficulty No. 4 proved insuperable and it was decided to dry as rapidly as possible to constant weight at 100° C. using a flat-bottomed flask, keeping conditions as far as possible constant throughout. After trials with several organic solvents, ether dried over anhydrous calcium chloride and sodium was finally found the most satisfactory, as it is very volatile at low temperatures, and is selective in extraction.

Apparatus. Soxhlet extraction apparatus was used, with Davis double jacketed condenser, flat-bottomed flasks, all the joints being of ground glass. The best form of heating apparatus is an electric hot plate, a thick asbestos sheet being interposed so that the ether only gently simmers, it should not be allowed to boil violently. The extraction flask could be readily converted into the Perkin methoxyl determination apparatus by means of a long accurately ground-in glass neck with side tube, down the centre of which ran a narrower glass tube for carrying CO_2.

METHOD PROPOSED FOR EVALUATING THE ROOT.

The finely powdered root is extracted with dry ether in the Soxhlet apparatus as described above, the extract dried at 100° C. and weighed to constant weight.

Hydriodic acid (S.G. = 1·7) previously heated to 120° C. in a current of CO_2 to free from H_2S is added in an amount appropriate to the weight of extract and 5–10 c.c. of acetic anhydride slowly run in and the methoxyl content determined by the Perkin method(13). Drying of the ether extract should not be too prolonged, as the extract itself is normally only very slowly acted upon by the hydriodic acid, prolonged drying at 100° C. tends to accentuate this. The determination of the methoxyl content usually takes two or three hours, depending upon the amount of the extract treated. Collected results are shown in Table II.

Table II.

Root	Moisture content %	Extract % on root	Extract mean % on moisture-free root	Methoxyl content % on ether extract	Alcohol extract after ether extract %
X	8·62	22·1	—	—	—
		21·4	23·7	14·7	—
		21·7	—	14·77	4·1
Y	8·52	17·89	—	14·02	—
		17·44	—	14·7	8·07
		17·80	19·2	—	—
		17·36	—	14·0	—
Z	9·57	7·10	—	13·43	11·8
		7·15	7·9	13·5	—

The difficulties of obtaining closely concordant results are illustrated in Table II, but the results are sufficiently near to indicate that the method will serve for standardisation if the root is to be employed as an insecticide. This will be dealt with under the discussion of the correspondence existing between these results and the insecticidal tests carried out upon these three specimens. The values obtained are undoubtedly affected by the fact that the method of extraction is not entirely selective. There are small and probably varying amounts of fats and fatty acids present, and it was impossible to free the roots entirely from attached particles of soil.

The amounts of non-toxic material precipitated by lead acetate can be estimated by extracting the ether-extracted powder with alcohol. Results are also shown in Table II.

Summary and Conclusions.

The toxic principles of *Derris elliptica* have been isolated and some of their more simple properties examined. A chemical method for evaluating the root has been outlined and a suitable extraction apparatus described.

1. The most important constituents of the root are a white crystalline derivative usually called "tubatoxin" and a resin or series of resins identical with the "derride" of Sillevoldt and the "tubain" of Wray. Besides these two, yellow crystalline derivatives and a liquid resin were isolated.

2. "Tubatoxin," the yellow crystalline derivatives and the resins contain methoxyl groups and these compounds appear to be inter-related. "Tubatoxin" by exposure to light, and by prolonged boiling with organic solvents is converted into three yellow crystalline products and a resin. This suggests that the "anhydroderride" of Sillevoldt may have been formed during the process of extraction and may not exist as such in the root.

3. The poisons from the root are readily extracted by means of organic solvents. 95 per cent. alcohol extracts them together with non-toxic derivatives. Benzene, dry ether, carbon tetrachloride are also good solvents for extraction purposes and have a selective dissolving action on the poisons. Petroleum derivatives are not suitable for complete extraction. Prolonged boiling with solvents may cause some loss of toxicity in the extracts owing to chemical change in the "tubatoxin." For economic purposes, benzene and its congeners or alcohol are probably the most suitable extraction reagents, provided the temperature of extraction is not allowed to rise too high.

4. The root may be evaluated by chemical means by extracting the dry root with dry ether and the genuineness of the extracts confirmed by the determination of the methoxyl content by the Zeisel method. Extracts from different deliveries varied between 7 and 22 per cent. and the content of CH_3O in the extracts between 13·5 and 14·7 per cent. A qualitative test for "tubatoxin" devised by Dr Durham is outlined on p. 5.

5. The amounts of the non-toxic constituents vary widely in different consignments. They seem to have some value as emulsifying and wetting agents. As the root however arrives in this country in a dry state in which the constituents have probably coalesced the use of foreign emulsifying and wetting reagents is necessary and for maximum efficiency the use of organic solvents for preparing highly dispersed suspensoids appears advisable.

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A QUANTITATIVE STUDY OF THE INSECTICIDAL PROPERTIES OF *DERRIS ELLIPTICA* (TUBA ROOT)

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(With 3 Text-figures.)

Prologue.

The work here described has its origin in a demand on the part of horticulturists for additional insecticides, a demand which during the war became more insistent owing to the high price and, often, scarcity of the few substances known to be of real use for spraying purposes. As originally planned, the investigation was to consist in a preliminary exploration of a wide range of possible substances, to be followed by a more detailed examination of those most likely to give practical success. This programme has been adhered to in the main, a number of substances including Yahoo Root, Mowrah Seed, seed of a Tephrosia, Serjania paucidentata, having been tested in a preliminary manner. Some departure from the original plan has, however, been made in regard to one substance, the root of the plant *Derris elliptica*, commercially known as Tuba Root, which has been investigated in somewhat greater detail than would have been called for in a mere preliminary survey. This departure was made partly because at the time when the work started a contact insecticide effective against caterpillars was specially needed and also because the earlier experiments indicated the desirability of developing methods on a substance available in relatively large quantities, which was the case in regard to *Derris*. The former reason naturally directed the choice of test insects towards caterpillars of various species, which have been retained throughout, and the point is mentioned to explain why poisons which appear to act essentially as contact insecticides have been tested chiefly on caterpillars and little on sucking insects such as aphides, against which contact insecticides are more usually needed.

With regard to the development of technique, the difficulties from the biological aspect have proved serious and justify some discussion (the chemical and physical problems are discussed on a later page).

In the first place it is clear that where the relative toxicity of a number of different substances has to be measured, the test insects ought in theory to be similar as regards species, age, health and environment; secondly, they should be available in very large numbers and this at all times of the year. It has not yet been possible to meet these conditions. The first series of experiments were carried out with caterpillars both bred from eggs and collected in the field, chiefly with those of the Cabbage White Butterfly (*Pieris brassicae*), the Lackey Moth (*Malacosma neustria*), the Buff-tip (*Phalera bucephala*), the Gooseberry Sawfly (*Pteronus ribesii*) and those of another Sawfly (*Phymatocera aterrima*). While experiments on these subjects were in progress attempts were also being made to find a species of which the caterpillars would (under artificial treatment) be available throughout the year.

Finally a Noctuid moth, *Hadena oleracea*, was chosen as a species likely to fulfil this condition. This common British insect, which under outdoor conditions is partially double-brooded, has recently established itself in tomato houses where it appears to breed continuously as long as the requisite heat is maintained. Owing to the kindness of Dr Lloyd (then of the Lea Valley Experiment Station) a large number of autumn pupae were obtained from the Lea Valley and were kept in an incubator at a temperature of 82° F., the atmosphere being practically saturated. Moths emerged freely after about six weeks and paired readily in cages 2 feet square kept in a greenhouse in which the temperature ranged from 60–70° F. Eggs were laid in abundance, hatched in about four weeks, and a large number of young larvae were thus obtained in winter and early spring. At this point difficulties arose, for although it proved possible to rear larvae in hundreds, the attempt to produce the thousands required was a complete failure owing to the high mortality when the larvae were about half grown. Contrary to expectation, difficulties increased with the advent of summer owing to the irregular behaviour of the pupae from the winter larvae, which only produced moths one or two at a time over a period running into many months. There were therefore seldom sufficient moths out at any one time to allow for fertile repairings. Comparatively few eggs were laid and the final result was a number of small broods of larvae, all of different ages and none large enough to be of use in testing.

The few moths obtained from these larvae showed evidence of de-

cadence, laying a large proportion of misshapen and infertile ova, and although the strain is still in being it is not proving of much use for experimental purposes.

Similar results as regards irregularity in emergence have been recorded with tropical insects and it would seem likely that in most species a rest at some period is essential and that it is taken whatever may be the climatic conditions. When such conditions are uniformly favourable to the active stage of a species then the rest is taken quite irregularly, so that it is not possible to observe definite broods or generations.

Experiments with *H. oleracea* not proving successful, trials were concluded in so far as this paper is concerned with Lackey larvae and silkworms, which were reared from the egg in large numbers specially for the purpose.

The production of a suitable species in sufficient numbers was, however, only one difficulty; the methods of applying the insecticide to the test subject also gave considerable trouble. The first method used and the most simple was ultimately adopted for all the experiments here described, and consisted in dipping each caterpillar for a definite period (10 seconds) in the liquid to be tested. The caterpillars were then placed in jars containing the foodplant, and a few pieces of filter paper to absorb any excess fluid introduced with the wet caterpillars, the results being checked periodically during the subsequent 48 hours. Naturally the liquids varied greatly in their wetting power, while equally the different species of caterpillar were more or less difficult to wet. Further, a hairy caterpillar such as the Lackey or Buff-tip retained the fluid for a much longer time than a smooth caterpillar. It has not yet been possible to eliminate these disturbing factors, and all that could be done was to add a surface tension reducer which gave as thorough a wetting as possible, Saponin—to which the larvae showed no marked reaction—being usually used for the purpose.

Taking these various factors into consideration, great numerical accuracy cannot be claimed for the biological side of the work, but nevertheless it is believed that from the qualitative point of view the results are substantially correct. Quantitatively they may be regarded as of a fair order of accuracy.

In all cases great care has been taken to draw conclusions only from those experiments of which the results were undoubted, and even at the risk of losing matter of interest trials were discarded whenever there was a suspicion that a disturbing factor had been introduced—as, for instance, when deaths from disease or the attacks of parasites occurred

in the main stock from which the trial subjects were selected. Deductions were based only on biological observations which were beyond dispute and may thus be regarded as fully justified.

Much yet remains to be done from the entomological standpoint, notably with regard to the physiological action of *Derris*, which would seem to involve problems of a nature not apparent in the case of such an insecticide as nicotine. The poisonous constituents of *Derris elliptica* are found to be relatively non-volatile, insoluble and solid. It might therefore be expected that their maximum efficiency can be secured only when special means have been taken to ensure that they are finely dispersed through the test fluid. On the other hand, from the physiological standpoint it is not altogether clear how non-volatile and insoluble solids function as contact insecticides, which seems to be the case. There has as yet been no opportunity for tackling either this or the other biological problems which have arisen in the course of the work, and a satisfactory conclusion to the whole investigation is to some extent dependent on their solution. It is felt, however, that the results already obtained should not be held over pending work on problems of a fundamental nature and in consequence this paper, which must be regarded as a "progress report," is published.

The entomological part of the research was carried out by Messrs Fryer and Stenton of the Plant Pathology Laboratory of the Ministry of Agriculture, the chemical side by Messrs Tattersfield and Roach of the Rothamsted Experimental Station. The insecticide tests were done conjointly.

We have pleasure in expressing our thanks to Mr Collin for much valuable preliminary entomological work.

Insecticide Tests.

Two different methods for estimating the insecticidal properties of the root and its various extracts were employed:

Method (1). A qualitative method carried out on a number of different insects and characterised by the comparatively small number of individuals used for each test. It consisted in grinding up the root, or the constituents of the root, with distilled water, dipping the insects for a period of 10 seconds into the resulting emulsion, mixture or filtrate, as the case might be, according to the point that required elucidation. In addition to this a few experiments were carried out in which soap and starch were incorporated with the mixture to act as a suspensory material. In this way information was obtained as to how the pure aqueous mixture

or solution reacted physically with the insect, as, for instance, how it wetted, and whether the film remained permanent on the body or contracted into globules, and, again, how readily the various types of caterpillars were killed when brought into contact with the aqueous mixtures or solutions of the poisons.

The liquids used differed greatly in their wetting power and the several species of caterpillars, although varying somewhat, were on the whole difficult to wet. In the earlier experiments the aqueous mixtures of ground root were found to possess wetting properties to a moderate extent, which seemed however to decrease somewhat in accordance with the age of the root. The extracted constituents showed the property of wetting to only a small degree, thus probably affecting the results profoundly. As a rule hairy caterpillars such as the Lackey and Buff-tip wet more easily than others, but this is not invariable, as the relatively glabrous larvae of the gooseberry sawfly were not difficult to wet. The results are set forth in Table I.

The table is divided up into several groups, Group I refers to experiments carried out with finely ground root *Z*[1]. It is obvious that the root merely macerated with water is fairly poisonous to a number of insects at a 2 per cent. concentration, although still toxic at 1 per cent. and 0·5 per cent. it is less so than at the higher concentrations, and an addition of soft soap does not greatly help matters. Autoclaving the root to a temperature of 120° C. slightly lessens its toxic properties. That the poison is not volatile to any great extent is evidenced by the fact that the distillate in steam is not toxic. Exp. 8 was carried out to see whether aqueous mixtures would lose their toxic properties on standing, and by allowing fungi and bacteria to grow freely in the liquid. This mixture stood for one month; the resulting material seemed less toxic to *P. brassicae*, but on the whole the diminution was not great. The extraction experiments in Group II were carried out, to find out the most convenient way of isolating the poisons. It is obvious that the organic solvents, particularly alcohol, do extract the poisonous material and leave an inert residue behind. Group IV indicates the toxic properties of the pure poisons isolated from the root. The fact emerges that these pure substances and extracts do not appear as poisonous as the emulsions ensuing from maceration of the root with water. In the case of the extracts this is readily explained by the fact that prolonged drying at 100° C. causes chemical change in some of the toxic constituents. The

[1] Three commercial samples of Derris Root labelled *X*, *Y*, *Z* for convenience were used in these experiments. Their derivation will be found on p. 13.

Table I.

Material used and Description of Test	Insects used: M. Neustria	H. padellus	P. brassicae	N. ribesii	P. aterrima	P. bucephalae
Ground root experiments (Group I)						
1. 2 % ground root macerated with water	×	×	×	×	.	×
2. 1 % „ „ „	.	.	×	×	.	.
3. 2 % air dried root (ground). Made up 20 hours and filtered	.	.	*	×	.	.
4. 2 % ground root, autoclaved to temp. 120°. Made up 20 hours and filtered	.	.	+	.	+	.
5. 0·5 % ground root. Made up 20 hours and filtered	.	.	*	*	*	.
6. Ground root distilled in steam. Distillate	.	.	○	.	.	.
7. „ „ „ Residue	.	.	−○	×	.	.
8. 2 % ground root aqueous extract, allowed to ferment	.	.	− −	×	×	.
9. 1 % ground root + ·5 % soft soap (*vide* No. 2)	○	.	.	.	.	.
10. 1 % ground root + ·8 % starch	○	.	.	.	.	.
11. 0·5 % soft soap	○	.	.	.	.	.
Extraction experiments (Group II)						
12. 2 % ground root No. 1 above (for comparison)	×	×	×	×	.	×
13. 1 % „ „ No. 2 „ „ „	.	.	×	×	.	.
14. 2 % of the alcoholic extract of ground root (free from alcohol)	+	.	.	.	.	.
15. 1 % „ „ „ „	○	○	.	.	.	.
16. Alcoholic extract (free from alcohol) later experiment	.	.	×	×	×	.
17. Residue from alcoholic extraction in No. 14	○	.	.	.	.	.
18. 2 % of carbon tetrachloride (CCl_4) extract	.	.	○	× ×	.	× ×
19. Alcohol extract of residue of 18	.	.	−	○*	.	*
20. Alcohol extract of ground root. No. 16 above	.	.	×	×	×	.
21. Pentane extract of alcoholic extract No. 20	.	.	.	×	×	.
Saponification experiments (Group III)						
22. Alcoholic extract before saponification. No. 16 above	.	.	×	×	×	.
23. „ „ after saponification with KOH	.	.	−	+	+	.
24. Unsaponifiable fraction of 23	.	.	*	*	*	.
25. Saponifiable fraction of 23	.	.	.	○	.	○
Experiments with derivatives from root (Group IV)						
26. 0·5 % yellow crystals. Mixture of tubatoxin and anhydroderride	.	.	.	×○	.	×
27. 0·5 % tubatoxin	.	.	.	×	.	+
28. 0·5 % anhydroderride	.	.	.	○	.	○
29 *a*. Derride. Resin from root. Not filtered	×	.	.	.	.	.
29 *b*. „ „ „ Filtrate from *a*	×	.	.	.	.	.
30. Oil (liquid resin) contains tubatoxin	.	.	.	.	.	×

The symbols employed in this table have the following significance:

× = 100 per cent. deaths.

\+ = Severe sickness clearly due to experiments, often with deaths.

* = Sickness definitely due to experiments.

○ = No effect.

− = Uncertain—no conclusions permissible.

case of the pure substances and their apparently lower toxicity is discussed below. The saponification experiments were carried out with the object of seeing whether this would result in loss of toxicity—it does so. It was considered possible that some slight reaction of this type might ensue upon mixing this root with alkalies or soaps. There is, however, little danger of this taking place at ordinary temperatures, particularly if the powdered root mixture is fairly dry.

Method (2). The main fact arising from Table I is that when tested by method No. 1 the pure products (Gp IV) derived from the roots do not appear so toxic as the emulsions (Gp I) prepared from the fresh root by mere maceration with water. This result may arise from a variety of causes.

1. There may be constituents in the root which maintain the poisons in a very fine suspensoid condition.

2. These emulsifying constituents may enable the mixture to wet and to spread over the body of the insect.

3. The constituents of the root may have undergone some chemical change in the process of extraction.

Points 1 and 2 are of considerable importance, but in the absence of precise physiological knowledge as to how Derris poisons affect insects they cannot be usefully discussed. It can only be pointed out that in whatever way the poisons obtain entry to the insect, the size of the particles present in the fluid and the wetting power must have a direct bearing on the efficacy of the poison as an insecticide.

Point No. 3 could be determined only by the elaboration of a more exact quantitative method for testing the root and its products. This involved a considerable amount of work on methods of suspending in water the solid poisons in as fine a condition as possible, in such a way that the materials from the root were the only effective toxic constituents present and any variation in that toxicity resulted from a change brought about in the physical condition of the poisons.

The following method for preparing finely suspended mixtures of poisons was adopted: an alcohol extraction of the root made in the cold was slowly poured into a solution of saponin in water, and the mixture freed from alcohol by distilling off under fairly high vacuum at a temperature not exceeding 35° C. For convenience the concentrations of these suspensoids are expressed in terms of equivalent root—that is to say, they contain as much poison as could be found in mixtures containing the stated percentage of root.

(The mixture froths somewhat when the alcohol is reduced below a critical amount, but, with care, loss due to this need only be slight.)

The pure poisons were dissolved in a minimum quantity of alcohol and treated in the same way, a rather larger amount of saponin being necessary to render the tubatoxin suspensoid permanent. In this manner suspensoids of known strength were prepared, and by the addition of saponin solutions of appropriate concentrations a whole series of mixtures of constant saponin content but varying progressively with respect to the amount of poison present could be readily made. It was our intention to use these mixtures as a spray, but as suitable apparatus was not available, a dipping method was again employed.

A known number of insects, usually five or ten, were immersed for ten seconds in each of a series of known dilutions, and then placed upon their normal food supply and kept under observation for a period of two to three days.

Table II.

Insect used: larvae of Tomato moth (*Hadena oleracea*). No. of insects per test—5. Dipped for 10 seconds. 1 % saponin used for *X* and *Z*, except those figures in brackets which refer to ·2 % saponin.

	Root *X* Ether extract = 21·8 %			Root *Y* Ether extract = 17·6 %			Root *Z* Ether extract = 7·1 %		
% of root	No. used	No. killed	No. partially affected	No. used	No. killed	No. partially affected	No. used	No. killed	No. partially affected
2·0	5	4	0	—	—	—	5	0	0
3·0	5+(5)	3 (2)	0	—	—	—	5	0	0
3·5	(5)	(4)	(1)	—	—	—	—	—	—
4·0	5+(5)	5 (4)	(1)	(5)	(4)	(1)	5	0	1
6·0	5	5	0	(5)	(3)	0	5	3	0
8·0	—	—	—	(5)	(4)	(1)	—	—	—
9·0	—	—	—	—	—	—	5	3	0
10·0	—	—	—	(5)	(4)	0	(5)	(1)	(1)
12·0	—	—	—	—	—	—	(5)	(1)	(1)
14·0	—	—	—	—	—	—	(5)	(1)	0
16·0	—	—	—	—	—	—	(5)	(1)	0

Dipping for 10 seconds in distilled water and 1 % solution of saponin was without effect.

Table II shows results obtained by dipping larvae of *Hadena oleracea* in suspensoids of the alcoholic extracts from roots *X*, *Y*, *Z*. The mean percentage of ether extract[1] is also included for purposes of comparison. The wetting was found to be good. Unfortunately, as only a limited number of these larvae were available, the results are not complete and we were unable to test out upon them the various pure constituents of the root. Controls with 1 per cent. saponin, a percentage never exceeded, and water alone were tested at the same time and proved to have no effect on the larvae.

[1] See p. 13 for method proposed for evaluating root.

Inspection of this table indicates that there is a difference in the toxic properties of the various consignments of this root. The results, although not complete enough to enable us to draw final conclusions, are in general agreement with those obtained in a similar manner using silkworms (see Table III). In the case of root *Z* it was found that on increasing the concentration beyond 9 per cent. a very imperfect suspensoid was obtained. This probably accounts for an actual decline in the proportion of deaths when these higher strengths were tested. Irregularities in the data show that sufficient numbers of insects have not been taken to eliminate the random variations in individual resistance.

Table III.

Toxicities to silkworms of different consignments of tuba root.

Ether extract of root *X* = 21·8 %. Root *Y* = 17·6 %. Root *Z* = 7·1 %. 1 % saponin. Insect used—silkworm. No. per test—10. Dipped five at a time for 10 seconds.

Description of test Root %		% of dry ether extract	No. dead	No. moribund	No. slightly affected	Total no. affected	Marks awarded
Root *X*	2·0 equivalent to	0·436	10	0	0	10	100
	1·0 „	0·218	10	0	0	10	100
	0·5 „	0·109	10	0	0	10	100
	0·25 „	0·0545	10	0	0	10	100
	0·125 „	0·0272	7	0	0	7	70
	0·025 „	0·00545	1	2	0	3	20
	0·0125 „	0·0027	1	0	0	1	10
Root *Y*	2·0 „	0·352	10	0	0	10	100
	1·0 „	0·176	10	0	0	10	100
	0·5 „	0·088	10	0	0	10	100
	0·25 „	0·044	8	0	0	8	80
	0·125 „	0·022	6	0	0	6	60
	0·05 „	0·0088	1	1	0	2	15
	0·025 „	0·0044	0	0	0	0	0
	0·0125 „	0·0022	0	0	0	0	0
Root *Z*	20–2 „	1·42–·142	10 each	0	0	10 each	100 each
	20–2 „	1·42–·142	„	0	0	„	„
	1 „	·07	9	1	0	10	95
	0·5 „	·035	6	0	4	10	70
	0·25 „	·0175	4	1	3	8	52·5
	0·1 „	·00875	0	0	0	0	0
Saponin solution (aqueous) 10 %		—	2	0	0	2	—
Ditto 5, 2, 1, 0·2 %		—	0	0	0	0	—
1 % saponin and alcohol 10 %		—	1	0	0	1	—
„ „ „ „	5	—	0	0	0	0	—
„ „ „ „	1	—	0	0	0	0	—
„ „ „ „	0·5	—	0	0	0	0	—
„ „ „ „	0·1	—	1	0	0	1	—
Alcohol solution 10, 5, 1, 0·5, 0·1 %		—	0	0	0	0	—

As there were available a large number of silkworms (*Bombyx mori*) free from disease, of known history, of about the same age and reared under exactly the same circumstances throughout, they were employed on a series of tests. The mixtures were made up and the tests carried out in the same way as described, except that ten insects were usually dipped, five at a time. The results are shown in Table III, Column 1 being expressed as described on p. 24. As the limit of toxicity is approached these results tend to become somewhat uncertain owing to variation in insect resistance. Many more larvae than we had at our disposal would have been required for the application of exact statistical methods and therefore to enable us to plot curves showing quantitative effects, different values were given to those which were killed and those partially affected. "Marks" were therefore awarded as follows:

A death was allowed ten marks.

A *moribund* condition five marks.

An affected but not *moribund* condition 2·5 marks.

The results were added up, calculated to a basis of 10 where less than that number of larvae had been used, and the sums of marks plotted against the amount of root or poisons employed in preparing our mixtures.

An inspection of Table III and Diagram I show very clearly that the ether extraction method (see Tattersfield and Roach, *ibid.* p. 13) is sound for purposes of evaluation. They indicate that root *X* is slightly but not very greatly superior to *Y* and that both of them are considerably superior to *Z*. The 50 per cent. death points are the most suitable for comparison. In Diagram II the results are presented differently. The toxicities of the different samples are calculated to their content of poison as estimated by the dry ether extract and these are plotted instead of the gross percentage of root; on the same diagram are plotted the results obtained in the same way with a sample of commercial nicotine of 92 per cent. strength (calculated to 100 per cent.).

It will be seen from Diagram II that a line can be drawn passing approximately through the various points that indicate the toxicities at the different concentrations of the samples *X*, *Y*, *Z*. The toxicity points of sample *X* are on the whole to the left of the line while those of *Y* and *Z* are on the right. Samples *Y* and *Z* both contain much more non-toxic material precipitated by lead acetate than does *X*, and a little of this would be extracted along with the poisons, as is borne out by the fact that the amount of methoxyl contents (see Table II, p. 15) of *X*, *Y* and *Z* descends in that order from 14·7 to 13·5. The results obtained are very close considering the difficulties encountered

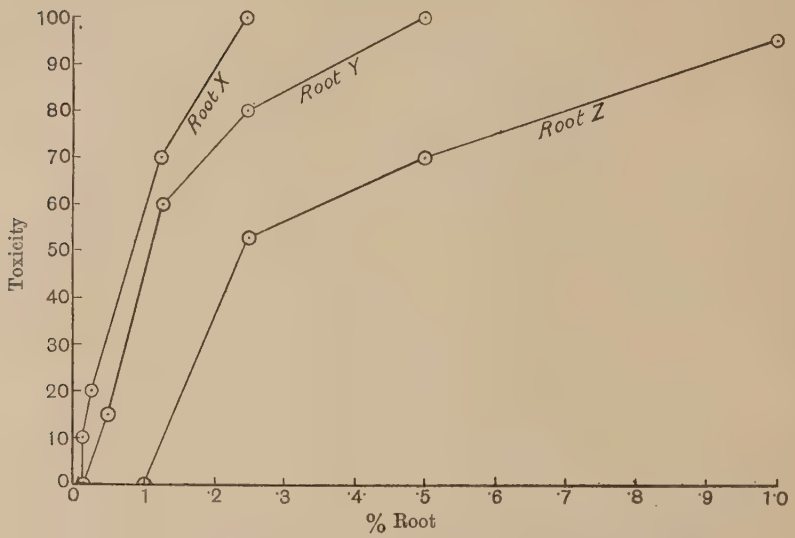

Diagram I. Showing toxicity to silkworms of different samples of tuba root.

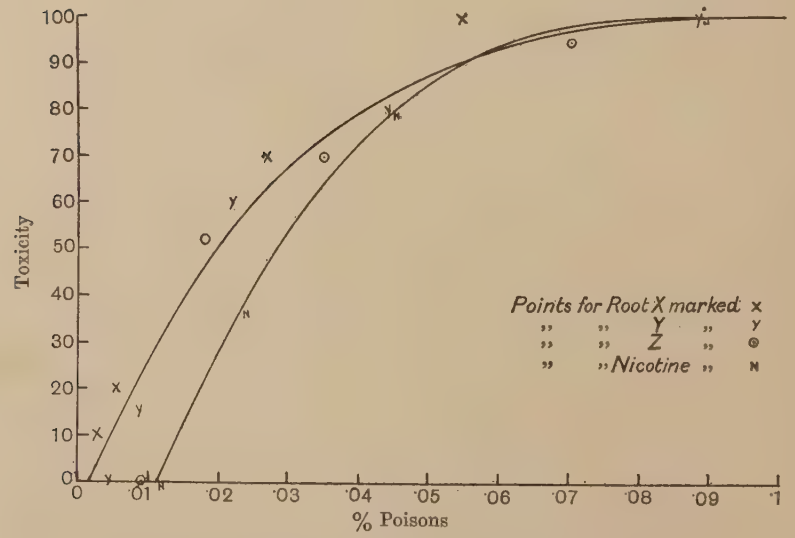

Diagram II. Showing relation between the toxicities of the various samples of root and the amount of ether extract.

in carrying out these insecticidal tests quantitatively. The diagram shows the extracts to be slightly but definitely more toxic than nicotine.

Table IV and Diagram III show the toxic relationships of the various pure products isolated from the root.

Table IV.

Toxicities to silkworms of products derived from tuba root and of nicotine.

No. used per test = 10 dipped for 10 seconds, 5 at a time. 1 % saponin.

Description of test	% poison	No. dead	No. moribund	No. slightly affected	Total No. affected	Marks awarded
Tubatoxin	0·1	10	0	0	10	100
Finely dispersed	0·05	10	0	0	10	100
	0·025	8	0	2	10	85
	0·0125	3	0	3	6	37·5
	0·005	3	0	2	5	35
	0·0025	3	0	0	3	30
	0·00125	1	0	0	1	10
	0·0005	0	0	1	1	2·5
	0·00025	0	0	0	0	0
Resin *A*, fraction 2, M.P. 72–82° C.	0·5	10	0	0	10	100
Precipitated by pentane from	0·2	10	0	0	10	100
ether solution	0·1	10	0	0	10	100
	0·05	6	1	0	7	65
	0·0125	1	1	0	2	15
	0·005	1	0	0	1	10
	0·0025	0	0	0	0	0
Resin *A*, fraction 3, M.P. 62–76° C.	0·5–0·2	10	0	0	10	100
Precipitated by pentane from	0·1	10	0	0	10	100
ether solution. Large excess of	0·05	10	0	0	10	100
pentane	0·025	8	2	0	10	90
	0·0125	6	0	4	10	70
	0·005	8	0	4	10	90
	0·0025	8	0	2	1	85
Resin *A*, fraction 4, M.P. 60–64° C.	0·5–0·2	10	0	0	10	100
Soluble in pentane and ether,	0·1	10	0	0	10	100
insoluble in pentane	0·05	10	0	0	10	100
	0·025	6	0	4	10	70
	0·0125	2	0	3	6	27·5
	0·005	0	0	1	1	2·5
	0·0025	0	0	0	0	0
Resin *B*. Different sample corres-	0·5–0·2	10	0	0	10	100
ponding to a mixture of *A* 3 and	0·1	10	0	0	10	100
A 4 more recently prepared, in-	0·05	9	0	1	10	92·5
soluble in a mixture of ether	0·025	9	0	1	10	92·5
1 vol., petroleum ether 2 vols.	0·0125	8	0	2	10	85
	0·005	4	0	1	5	42·5
	0·0025	2	0	0	2	20
	0·00125	0	0	0	0	0
	0·0005	2	0	0	2	20
Nicotine	0·092	10	0	0	10	100
	0·046	8	0	0	8	80
	0·023	3	1	1	5	37·5
	0·0115	0	0	0	0	0
	0·0046	1	0	0	1	10
	0·0023	0	0	0	0	0

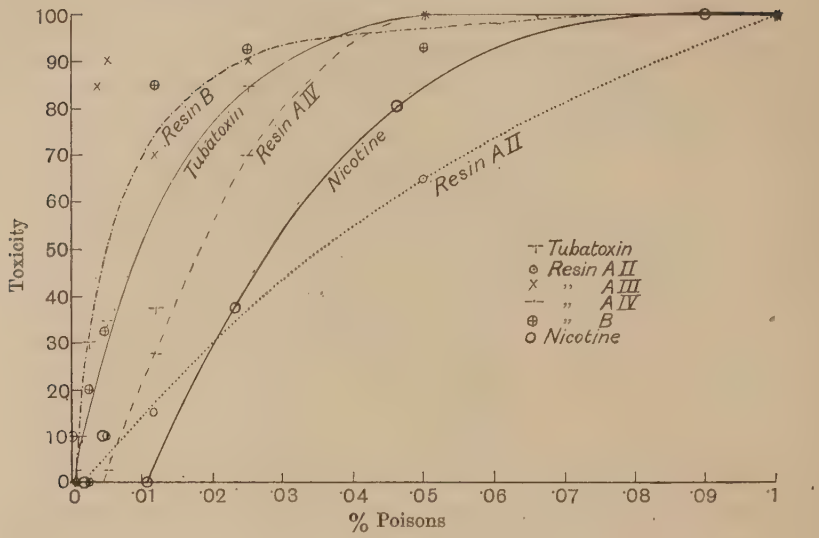

Diagram III. Shewing toxicities of constituents of tuba root in comparison with that of nicotine.

Points for Resin AIII are too irregular for a curve drawn through them to have meaning.

Table IV and Diagram III indicate that the pure products tubatoxin and the various fractions of the resin derride have a toxicity of an order comparable with that of nicotine. Details of fractions of derride *A* 2, 3 and 4 have been given on p. 12. Fraction *A* 1 was too small in quantity to be tested. Fraction 3 more closely corresponds to the resin discussed by Sillevoldt and Greshoff, than the others, while sample *B*, a resin

Table V.

Showing effect of filtering off suspensoid.

Insect used—silkworm. No. of insects per test—10.

Description of test	% of root	No. dead	No. moribund	No. slightly affected	Total No. affected	Marks awarded
Root *Z*	20–2	10	0	0	10	100
	1	9	1	0	10	95
	0·5	6	0	4	10	70
	0·25	4	1	3	8	52·5
	0·1	0	0	0	0	0
Filtrates from the above	from 20	7	3	0	10	85
	„ 16	6	3	1	10	77·5
	„ 12	8	1	1	10	87·5
	„ 8	8	1	0	9	85
	„ 4	1	0	2	3	15

prepared at a later date is analogous to a mixture of fractions 3 and 4. All these products were tested in a very finely suspended condition.

The effects of filtration on the toxicities of the suspensoids in water of the alcoholic extracts of one of the roots (Z) is shown in Table V.

Before filtration the suspensoid phase consists of particles ranging continuously from those of microscopic to those of molecular dimensions. An unknown quantity of particles of the sub-microscopic size would pass through the filter paper (No. 1 Whatman), but the total amount of the latter was relatively minute. After filtration the fluid still had a high toxicity. This fact seems to justify the belief that a more exact study of the relationship between fineness of dispersion and toxic action may lead to a more fundamental knowledge of the economic preparation of spray fluids. A tentative conclusion may be drawn from these facts, that for maximum efficiency the poisons of a spray fluid, when solid and non-volatile, must be present in as finely a divided suspensoid condition as possible. Early investigators noted that the fresh samples of root

Table VI.

Toxicities to silkworms of resins prepared from tubatoxin.

No. of insects per test—5. 1 % saponin.

Description of test	% poison	No. dead	No. moribund	No. slightly affected	Total No. affected	Marks awarded
Artificial resin No. 1 precipitated by pentane from solution in ether	0·5	5	0	0	5	100
	0·4	4	0	1	5	85
	0·3	5	0	0	5	100
	0·2	5	0	0	5	100
	0·1	3	0	0	3	60
	0·05	2	0	0	2	40
	0·025	0	0	0	0	0
	0·0125	1	0	0	1	20
	0·0005	1	0	0	1	20
	0·00025	0	0	0	0	0
	0·000125	1	0	0	1	20
Artificial resin No. 2 soluble in pentane and ether	0·5	5	0	0	5	100
	0·4	4	0	1	5	85
	0·3	5	0	0	5	100
	0·2	3	0	0	3	60
	0·1	1	0	0	1	20
	0·05	2	0	0	2	40
	0·025	1	0	2	3	30
	0·0125	0	0	0	0	0
	0·005	1	0	0	1	20
	0·0025	0	0	0	0	0
	0·00125	1	0	0	1	20

had higher toxic properties than those which had been long stored. Some indication of this was also observed in the present work, and the cause appears to be the drying up of the sap and the consequent coalescence of the poisons. Poisons in the milk-like product, resulting from the maceration of the fresh root are in a fine suspensoid condition, whereas with the aged root this is not the case and special means have to be adopted to achieve it.

Table VI shows the relationship between the toxicities of the resin produced by the prolonged boiling of tubatoxin with alcohol.

This reaction gives rise to two series of products:

(1) Crystalline derivatives (anhydroderride).

(2) Resin. This resin melts at 62–72° C. It can be fractionated to some extent by the precipitation of its ether solution by pentane.

The crystalline derivatives do not appear to have any toxic properties but unfortunately we did not have opportunity to test them in a finely divided condition. It was desirable to ascertain whether the resin derived from tubatoxin was poisonous or not, in order to discover whether any loss of toxicity was likely to arise from the action of boiling alcohol, if it were used for purposes of extraction.

If comparison is made with tubatoxin and resin *B* (derride), the artificial resin is distinctly less toxic than either. It appears therefore that prolonged boiling with solvents in process of extraction is not desirable.

Aphid Spray Trials.

Durham found aphides (*A. rumicis* and *E. lanigera*) had a considerable resistance to derris root and its products. McIndoo, Sievers and Abbott state that aphides sprayed with derris mixtures and extracts, behaved almost normally and showed no symptoms of paralysis, and that they died very slowly. Our experiments upon Bean Aphis tend to confirm these conclusions. Broad beans heavily infested with aphis were sprayed with emulsions of the root extract of strengths of 4, 2, 1, and 0·5 per cent. of the root. The toxic action was extremely slow and uncertain, while nicotine oleate in a dose containing ·05 per cent. nicotine was immediately and almost completely effective. This result on so vulnerable an insect is not easy to understand.

The wetting power of emulsions prepared from different consignments of root.

A few tests were carried out to ascertain if the non-toxic constituents of the root had any effect upon the wetting and spreading powers of its

emulsions. Roots *X* and *Z* were tested. They were ground up with sand and water, made up to the required bulk in bottles, mechanically shaken for 20 hours, the coarsest particles allowed to settle and the turbid liquid decanted off for testing purposes.

Root *X* did not give a foam when shaken, *Z* however did, but it was not of a very permanent nature.

The tests were carried out as follows: five larvae of *Hadena oleracea* were dipped in different dilutions of these emulsions and a note made of the permanence of the film on the body. The results are shown in Table VII.

Table VII.

Effect of non-toxic materials on wetting properties.

Insect used—*Hadena oleracea*.

Test No.	Description	% of root present	% of non-toxic extractives present	% of toxic extractives present	Wetting properties, etc.
119	Root *X*	10	·41	2·2	Good—Continuous film
120	„	5	·20	1·1	Film broke
121	„	2	·08	·45	„ „
122	„	1	·04	·22	„ „
123	Root *Z*	10	1·18	·71	Very good—Continuous film
124	„	5	·59	·35	„ „
125	„	2	·29	·17	„ „
126	„	1	·14	·08	„ „

These results indicate that while toxic materials have little or no effects on the wetting and spreading powers of the extracts, some constituent of the non-toxic materials has, aqueous extracts of root *Z* showing distinctly better results in this respect than *X*. In none of the roots, however, were the inherent wetting and spreading properties sufficiently good to warrant reliance being put on them alone. For spraying purposes the addition of foreign wetting and spreading agents is essential if maximum efficiency is to be obtained, particularly when the dry powdered root is used.

Summary and Conclusions.

1. Extracts of *Derris elliptica* are shown to have a high insecticidal value, particularly for caterpillars. They are not so toxic to aphides.

2. The principles of the root toxic to insects are the white crystalline derivative, usually called "tubatoxin," and a resin of a golden yellow colour identical with the "derride" of Sillevoldt. Both these compounds when finely dispersed in water have considerable toxic properties to insects.

3. The dry root itself may be used in a finely powdered condition worked up with water together with soap or other emulsifying reagents.

4. As the pure poisons found in derris root are solids and only slightly soluble in water, their toxicity appears to depend upon their degree of dispersion.

5. A biological method of determining insecticidal properties quantitatively is described. It depends on dipping insects for a constant period of time in known strengths of highly dispersed suspensoids in dilute aqueous solutions of saponin. Results agreeing with those given by the chemical method already described (p. 13) were obtained. It enabled us to compare extracts of derris with nicotine. To certain caterpillars tubatoxin and derride are shown to be of the same order of toxicity as nicotine.

(*Received February 22nd*, 1922.)

BIOLOGICAL STUDIES OF *APHIS RUMICIS* LINN.

THE PENETRATION OF PLANT TISSUES AND THE SOURCE OF THE FOOD SUPPLY OF APHIDS[1]

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(With Plates I, II and 4 Text-figures.)

CONTENTS.

I. Introduction.

The food of aphids and the means by which it is obtained from the plant host, is an aspect of the biology of these insects which has been very little studied. Büsgen(6) was the first to seriously investigate the problem, more especially in relation to the production of "honey dew." Petri(15, 16, 17, 18) studied the question in the case of the vine *Phylloxera* and his researches have considerably advanced our knowledge of the subject.

In 1914 Zweigelt published his investigations on the subject and this paper, together with his later work on aphid galls in 1916, are important contributions to the literature.

Some smaller papers have been published but they are of a superficial nature and as they are, with few exceptions, referred to in the papers quoted above it is unnecessary to review them here.

[1] A grant in aid of publication has been received for this communication.

The investigation is naturally largely a botanical one, demanding particularly a knowledge of plant cytology and vegetable physiology. The present writer therefore has attempted to describe actual observations, rather than to enter into a discussion of the numerous theories which naturally suggest themselves as an explanation of the problems which arise. It is felt that further research on the relationship existing between the host plant and aphids, especially as regards the physiological and chemical changes occurring in the plant, and the physiology of digestion in aphids, is necessary before a clear explanation of the problems can be established. An investigation of the subject is desirable in view of the increasing importance of aphids as possible vectors of certain widely spread diseases in plants.

My thanks are due to Dr A. D. Imms for his helpful criticism during the preparation of this paper.

II. Technique and Methods.

The following aphids have been used in the investigations: *Aphis rumicis* L. on *Rumex*, *Vicia faba*, *Euonymus europaeus*, and *Chenopodium album*. *Macrosiphum rosarum* Walk. on rose trees and *Myzus cerasi* F. on peach.

Material was fixed with Farmer's fluid (absol. alc. 2 parts by volume and 1 part glacial acetic acid), or hot corrosive sublimate mixture (corros. subl. 3 gms., glacial acetic acid 3 gms., alcohol 60 per cent., 100 c.c.).

Small pieces of the infested plant were cut off quickly and allowed to fall direct into the fixative. In this way as many as 95 per cent. of the aphids were fixed with their stylets *in situ*.

Boiling water was useful for studying phloem contents. The infested part of the plant required was first bent over into 80 per cent. alcohol and immersed for a few seconds in order to stupify the aphids. It was then plunged into a large vessel of boiling water and the required parts quickly cut off *under* the water and allowed to fix. Air bubbles frequently enter the xylem vessels if the pieces are not cut off *under* the water and prevent proper fixation.

After fixation, the material was carried through in the usual way to the paraffin embedding bath. Great care must be exercised in handling the material in the various media or most of the aphids will fall off. Sections were usually cut 10μ thick, sometimes thicker for special preparations.

The following stains were used:

Safranin and Delafield Haematoxylin.
Safranin Gentian-Violet orange G, triple stain.
Safranin and Lichtgrun (in clove oil).

The safranin formula used was, equal parts of saturated aqueous and saturated alcoholic solutions, the stain being allowed to act for 12 to 36 hours. Methylene blue, carbol fuchsin and ruthenium red were also used for certain preparations as indicated in the text.

Eau de Javelle and Schweitzer's reagent were used for certain purposes as well as other reagents which are referred to in the text.

III. THE MOUTH-PARTS AND THEIR RELATION TO THE PHENOMENON OF SUCTION.

The mouth-parts and mechanism of suction in aphids has previously been described by the writer(8). Awati(1) also described them in the Capsid bug *Lygus pabulinus* Linn., and recently Grove(11) has dealt with

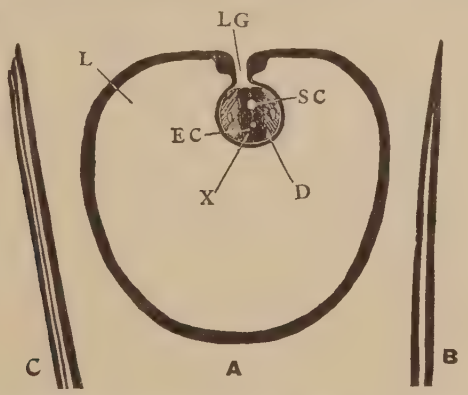

Fig. 1. A. Transverse section through the piercing organ of *A, rumicis* near the tip of the labium. The stylets composing the piercing organ are seen lying in the labial groove. L=labium; X=maxillary stylets; D=mandibles; LG=labial groove; SC=suction canal; EC = ejector salivary canal.
B. The pointed extremity of a mandible.
C. Side view of the extremity of the maxillary stylets showing the suction canal and ejector salivary canal. × 540.

the same subject in *Psylla mali*. These authors have also reviewed the literature on the subject and the reader is referred to the above papers for detailed accounts.

Briefly, the parts associated with the mechanism of suction are the "forehead," which is composed of the clypeus and labrum, the labium or proboscis, a pair of mandibles and a pair of maxillary stylets.

In aphids the mandibles and maxillary stylets are pointed at their distal extremities (Text-fig. 1). In repose they lie closely apposed in the labial groove (*l.g.*) and function as one stylet, which I shall refer to in this paper as the piercing organ. It is this organ which penetrates into the plant tissues.

The internal or maxillary stylets (*x.*), on emerging from the head, become permanently closely apposed, thus forming two canals which lead to the extremity of the stylets (Text-fig. 1).

The dorsal and larger canal is the suction canal (*s.c.*) which carries the plant juices directly into the pharyngeal duct and so into the pharynx, from which they are forced back by the muscular action of the pharynx, along the oesophagus, into the stomach. The ventral one is the ejector salivary canal (*e.c.*). This canal is continuous with the efferent salivary duct and thus conveys the salivary secretion from the chamber of the salivary pump into the plant.

The mandibles lie close to the maxillary stylets, but sometimes separate from them when the piercing organ is withdrawn from the plant, and thus apparently three stylets may be seen.

A modification is necessary of my explanation in the paper referred to above, as to the manner in which the piercing organ is forced into the plant. The pincer-like action of the labium in assisting the penetration and withdrawal of the stylets in coccids, described by Berlese (2, p. 152; 3, p. 187) escaped my notice. This feature has been recently described in detail by Grove (11) in *Psylla mali.*

A similar action of the labium takes place in aphids, the penetration and retraction of the piercing organ being further assisted by the movements of the "forehead" and by the partial retraction and protraction of the labium.

When one examines *Aphis rumicis* killed *in situ* with the stylets inserted in the plant tissues, the labium is seen in many cases to be much shorter than normally. The basal portion is in fact withdrawn in a postero-ventral direction, somewhat like the inverted finger of a glove, a feature which the writer (7) has also described in *Eriosoma lanigera* Hausm. The labial groove at its distal end completely enfolds the piercing organ, thus holding it in position. The further the piercing organ is inserted into the plant, the further is the labium retracted. Usually however only about one half to two-thirds of the labium is withdrawn, but its posteriorly directed position, together with the forward movement of the freely articulating "forehead," allows the maximum amount of the piercing organ to be inserted into the plant.

I believe there is actually very little independent protraction or retraction of the maxillary stylets themselves, apart from the combined movements of the "forehead" and the protraction and retraction of the labium. Such independent movements would dislocate the delicate mechanism whereby the pharyngeal duct and efferent salivary duct fit respectively into the suction canal and ejector salivary canal (*vide* Davidson(8), Plate XXIV, figs. 1 and 9). The mandibles, on the other hand, are capable of independent retraction and protraction, which probably assists in the penetration of the tissues.

Aphis rumicis was observed feeding under the binocular microscope. When individuals were disturbed by touching them gently with a small brush, they usually made energetic efforts to draw out the piercing organ from the plant. The head was vigorously used in a tugging manner, and the labium became apparently longer. Eventually, when the stylets were withdrawn, they were seen to extend some distance beyond the tip of the labium, but the latter organ soon regained its normal length and automatically the stylets occupied the position of repose in the labial groove.

IV. The penetration of the plant tissues by the stylets.

The piercing organ resembles a flexible bristle and is readily bent in any direction. Its course in the plant tissues is associated with the search after nutritive cell sap, as is well illustrated by the richly branching stylet tracks found in the phloem. In cleared preparations of a piece of stem or leaf with aphids *in situ*, it can be seen passing through the epidermis into the deeper tissues. In serial sections however, it is only occasionally that a good length of the piercing organ can be seen in one section. This is due to its irregular course through the tissues, which results in only small pieces being obtained in the sections. Frequently, these small pieces of chitin tear the section and drop out. Plate I, figs. 1 and 2 and Plate II, fig. 4 show the piercing organ in the plant tissues.

Fortunately it is easy to demonstrate the course taken by the piercing organ because, when this structure is in the plant tissues, a tube-like sheath, the stylet sheath, forms round it (Plate I, fig. 1). The contents of the latter, when the piercing organ is withdrawn, stains readily with certain stains, especially safranin, forming a definite track, the *stylet track* (*vide* Text-figs. 2, 3, and 4).

Sometimes the stylet track follows an irregular course in comparison with that of the actual piercing organ. This is doubtless due to three causes. (1) The dissolving action of the saliva. (2) The changing turgidity of the plant cells, which when the stylets are withdrawn squeezes

the tract irregularly. (3) The action of the fixative which often causes shrinkage of the tissues, owing to the comparatively large pieces which have to be fixed.

Plate II, fig. 3 shows the direct course taken by the piercing organ.

When a piece of a heavily infested plant is fixed, it follows that many of the stylets fall out during the subsequent technique. There are therefore numerous stylet tracks which may not show in the sections, owing to the fact that they are empty and do not stain.

Furthermore, owing to the chemical changes which take place as a result of the penetration of the tissues, the action of the saliva and the action of oxidases, some stylet tracks stain faintly or not at all.

The filling of the stylet sheath, after the piercing organ is withdrawn, is doubtless produced by a welling up of the plant juices owing to the osmotic pressure in the tissues[1]. It is seen in Plate I, fig. 3, for example, that the contents of the stylet sheath have been forced out on to the outer surface of the epidermis. The nature of the stylet sheath will be considered more fully in a later paragraph.

With *Aphis rumicis* the piercing organ enters between the epidermal cells and usually follows an intercellular course in the cortex, although occasionally individual cells may be pierced through (*vide* Plate I, fig. 3; Plate II, fig. 2 and Text-fig. 3).

In young meristematic tissue and in tissues where intercellular spaces are absent, the penetration may be intracellular, although the course is usually along the line of the cell walls. *Vide* Text-figs. 2 and 3.

The intercellular course in the loose cortical tissue, clearly shown in Plate II, fig. 2 is I think the line of least resistance for the piercing organ. The resistent turgid cells would tend to deflect this delicate structure while, on the other hand, when passing between the cells, there is considerable lateral support for it and its fine needle point makes penetration comparatively easy. Furthermore, it appears clear that the salivary secretion is able to digest certain cell walls, more especially the middle lamella, thus greatly assisting the penetration process.

In some cases, especially when the plant is heavily infested, one can see the cells are forced apart by the passage of the stylets, and in others, they are torn and destroyed by the combined physical action of the stylets and the effect of the saliva. The effects of the stylets and of the salivary secretion on the cells will be discussed in a later paragraph.

With regard to the passage of the piercing organ between the cells, the high pressure exerted by the latter under osmotic influence or turgor,

[1] See footnote on page 42.

must be considered. It will be seen however (Plate I, fig. 3) that the saliva coming in contact with the cell contents, causes extensive plasmolysis of the cells, thus reducing the pressure. Even where extensive plasmolysis does not take place, the osmotic pressure of the cell is I think not a serious difficulty, owing to the delicate nature of the piercing organ and the action of the saliva. The importance of the passage of the piercing organ along the line of the cell walls is, that support is thus afforded it during penetration of the tissues.

Büsgen (6) classified the course taken by the stylets in plant tissues, into three distinct types. (1) A course leading to the vascular bundles, passing intercellularly through the cortex. (2) A course passing intracellularly through the cortex and not going to the vascular bundles. (3) A course leading to the vascular bundles passing intracellularly through the cortex.

It is difficult to sharply demarcate these three types of stylet tracks. Certainly types 1 and 3 may be classed together as one type. With *Aphis rumicis* the first is the usual type met with.

Type 2 on the other hand does occur in some cases. Petri, having shown that *Phylloxera vastatrix* on the vine may not reach the vascular bundles, contenting itself with the cortical cells, through which the piercing organ passes intracellularly.

Zweigelt (26) considers that the intercellular passage of the stylets is associated with an extensive intercellular suction, which is the principal method by means of which the cell sap is obtained.

My observations lead me to the view that in the cases investigated, intracellular suction is the most important method of obtaining nourishment, and that the significance of the intercellular passage of the piercing organ through the cortical tissues, is associated with easier penetration on its way to the vascular bundles. Although there is frequently a marked plasmolysis of the cells bordering intercellular spaces in which saliva has been secreted, this is not always the case, and only occurs when the cell wall is so affected that the saliva comes in contact with the cell contents. On the other hand, most sections show stylet tracks ending in individual cells.

The intercellular spaces contain chiefly water and dissolved gases. In the case of intercellular suction, the cell membrane would exert a selective influence on the passage of nutrient substances from the cell into the intercellular spaces. Of course in cases where the protoplasm of the cell is killed by the action of the saliva, its selective power would be destroyed and the case would be almost equivalent to a case of intra-

cellular suction. This does sometimes happen, but often only a small quantity of saliva is found in the intercellular spaces, *vide* Plate II, fig. 2. The nature and occurrence of these irregular masses of saliva in the intercellular spaces, suggests that they are sometimes formed after the piercing organ has been withdrawn, by the pressure of the cells forcing the fluid along the stylet track, from between the cells.

One frequently sees a group of cells affected by the saliva, for example in the cortex of rose stems and in the phloem of peach tissue attacked by aphids (*vide* Plate II, fig. 1).

It is seen from the foregoing account, that with *Aphis rumicis*, the piercing organ follows a clean course (usually intercellular in the cortex, but occasionally passing through individual cells) to the vascular bundles, from the phloem elements of which it chiefly derives its nourishment.

V. The stylet sheath.

As the piercing organ penetrates into the tissues, the insect pumps saliva into the plant and the stylets thus become surrounded with plant juices and saliva. Owing to the action of the saliva, a precipitation or coagulation of substances results, which forms round the piercing organ a thin walled tube or sheath, the stylet sheath, Plate I, fig. 1, Plate II, fig. 4, which becomes irregularly thickened by a deposition of tannin[1]. When the piercing organ is withdrawn, it leaves a track in the tissues as explained on p. 39, which when stained with safranin, shows in sections as a definite reddish track, the stylet track.

[1] The primary wall of the stylet sheath is probably of a cellulose nature, but as can be seen in Plate I, fig. 1 there is an irregular deposition of substances on its outer surface. Petri, 1908, p. 28 and 1911, p. 58 considers that the sheath is composed in *Phylloxera* of callose and insoluble calcium pectate, tannin being deposited some time after the actual wall of the sheath is formed. This author considers that the contents of the sheath is largely saliva, but it seems to the writer that watery solutions of a non-colloidal nature pass through the wall of the sheath and thus mixing with the saliva give the well defined stylet track as explained in the text. It is probable that the brown coloration often seen in the cells bordering the older stylet tracks is due to oxidase action.

The presence of tannin along the stylet track is considered by some authors as representing an attempt on the part of the plant to resist the attack of the insect. Thus in the case of aphids on rose trees, there is frequently a callose thickening of the walls of the cells attacked, being a reaction on the part of the cell, to the influence of the saliva. The nature of the ferment action of the saliva needs further study. According to some authors its action results in a large accumulation of soluble carbohydrates in the areas affected by the saliva. Tannins may probably be formed in the presence of excess of soluble carbohydrates. Naturally owing to the state of our knowledge these views are largely theoretical.

Sections of bean stem were tested for soluble carbohydrates by treating them with a few drops of 10 per cent. solution of thymol in 50 per cent. alcohol, followed by a few drops of concentrated H_2SO_4. The stylet sheath showed as a delicate, colourless tube passing intercellularly through the cortex. When treated with iodine in K.I., followed by concentrated H_2SO_4, the stylet track showed brownish yellow in contrast to the blue cellulose reaction of the cell walls. Eau de Javelle destroys the stylet sheath. When treated with Schweizer's cupra-ammonia reagent and stained with carbol fuchsin, the stylet track shows clearly passing along the middle lamella. This is especially clear when the track passes through the walls of the thickened cortical cells situated at the four angles of the stem in *Vicia faba*. Sections treated for 24 hours with 22 per cent. HCl in 95 per cent. alcohol and afterwards stained with aqueous methylene blue, show the stylet track very distinctly in relation to the cells.

The staining action of methylene blue, following treatment with HCl as indicated above, suggests the presence of pectic substances. Petri's researches on *Phylloxera vestatrix* referred to in the footnote on p. 42, shows that the sheath is due to a precipitation of substances formed by the reaction of the saliva on the cells, and consists of callose and insoluble calcium pectate, having on its outer surface a deposition of tannin.

It is evident that a generalisation as to the chemical composition of the stylet sheath in aphids cannot be made. It is probably dependent on the species of aphis and plant concerned. It may for instance differ in the case of gall forming and non-gall forming aphids. In this respect it is interesting to note that Petri was unable to stain the stylet sheath in the case of *Mytilaspis fulva* Targ. on the olive.

Büsgen considered that the stylet sheath acts as a supporting structure for the stylets. This function must be a relatively unimportant one, owing to its delicacy compared with the piercing organ. Furthermore, the stylets are well provided with strengthening grooves and ridges on their internal faces.

In some cases the stylet track may be seen completely investing a cell. This does not mean that the piercing organ has curved round the cell, but indicates the dissolving action of the saliva, following the middle lamella of the cell wall.

The stylet track does not always stain. This probably depends upon the chemical changes which may go on after the piercing organ is withdrawn.

VI. The effects of the stylets and salivary secretion.

Owing to the needle-like extremities of the mandibles, the physical damage to the cells is not so extensive with aphids as with some other Rhynchota. For example in Capsid bugs the mandibles are serrated at the tip and considerable laceration of the tissues results. With *Macrosiphum* on the rose and *Myzus* on the peach, the cortical cells are often torn. In the case of *Aphis rumicis* on the bean however, the piercing organ takes a clean course through the tissues, and although plasmolysis of the cells occurs, there is very little tearing of the cells.

The salivary secretion exerts a diastatic action on the insoluble carbohydrates of the plant, changing them into soluble sugars; but in this respect the influence of diastase present in the plant itself, on the carbohydrates in the cell sap round the apex of the piercing organ, must be considered.

The salivary glands of *Aphis rumicis* were placed on an agar plate impregnated with starch, and after a time the plate was treated with iodine. In three cases, the areas where the salivary glands had been placed, showed white against the otherwise blue background of the plate, owing to the conversion of the starch by the action of the saliva. The diastatic action of the saliva is well established in Rhynchota in general. Petri has shown with *Phylloxera* that starch is converted into sugar in the affected cells, and similarly Zweigelt showed with certain aphids that there is an increase in sugars along the stylet track. It is of interest to notice that the starch grains (*G*) in Plate II, fig. 4 near the piercing organ, are smaller than those lying further away. This is one of the most important functions of the saliva, but the fact that saliva is frequently secreted, immediately the piecing organ enters the epidermis, suggests it is also important in assisting the penetration of that structure, by digesting the cell walls[1]. The remains of the salivary secretion is seen in Plate I, fig. 2 (coloured red), lying in the intercellular spaces bordering the piercing organ, near to the epidermis.

It would seem therefore, that the presence of a thick cuticle on certain plants may be of importance in preventing newly hatched aphids from piercing through to the underlying tissues. Cutin is notoriously resistent to the action of ferments and the initial piercing of the cuticle is probably a physical process. Older and stronger aphids might pierce the cuticle, but the presence of a thick cuticle suggests that the plant in general

[1] The cell wall is composed of cellulose in which a number of organic compounds may be deposited, forming the so-called hemi-celluloses, of which pectic substances are common.

would not be attacked, although colonies of aphids might develop on the young growing shoots.

When the saliva comes in contact with the cell contents, there is a marked plasmolysis of the cell and usually a migration of the protoplasm towards the source of the irritation, Plate I, fig. 3. The contents of the cell become disorganised, the nucleus swells considerably and eventually becomes a deeply staining, irregularly shaped, homogeneous mass. The cytoplasm becomes disintegrated and in extreme cases only a small layer of cytoplasm is left in the cell. The cells which are attacked eventually go brownish in colour presumably owing to oxidase action.

Even the lignified xylem vessels are acted upon by the saliva, the penetration of which is seen in Plate I, fig. 4.

The extent to which the local area attacked influences the surrounding tissues by a general distribution of the irritating salivary secretion is an important question. With *Aphis rumicis* there does not appear to be any spreading. It is more serious however in the case of *Myzus* on peach where many of the cells in the course of the stylet track are frequently torn and destroyed, Plate II, fig. 1.

Zweigelt found with *Aphis grossulariae* on *Ribes aureum*, that the cells were affected three or four rows away from the actual stylet track, and there was marked plasmolysis.

The spreading of the damage from the actual area penetrated, is seen more readily in some other Rhynchota, as for example in *Tomaspis saccharina* Dist. on the sugar cane, *vide* Williams (22).

Although the actual piercing through of the plant cells will eventually result in the death of those cells, the important factor is undoubtedly the action of the saliva. Smith (20) has demonstrated by experiment the harmful effects on plant tissues, of the saliva of certain Capsid bugs.

In sections of aphid-infested rose stems and also peach stems, one sometimes finds an irregular thickening of the walls of the cells in a badly infected area, due to the reaction of the cell under the influence of the saliva. Petri observed similarly with *Phylloxera* on the vine, a callose-like thickening of the walls of some of the affected cells. Zweigelt also observed a cellulose thickening of cortical cells in the rose stem. He also records that a similar feature, owing to the action of fungal hyphae (*Ustilago maydis*) has been observed by Guttenberg.

From the foregoing account, it will be readily seen that the plant exhibits many pathological features as a result of aphid attack, particularly owing to the influence of the salivary secretion. However it must

not be overlooked that once the physiological relationships existing inside the cell are disturbed, owing to the influence of the saliva, the complex chemical changes which may result, cannot at the present stage of our knowledge be clearly understood. Further research is necessary on the action of the saliva of different species of aphids on different plant hosts and the changes which occur in plant tissues infected with salivary secretion. Further, the physiology of digestion in aphids requires to be investigated.

VII. Sources of the Food Supply.

The food of aphids is the cell sap of plants derived from various cells of the plant tissues, especially the vascular bundles. The numerous

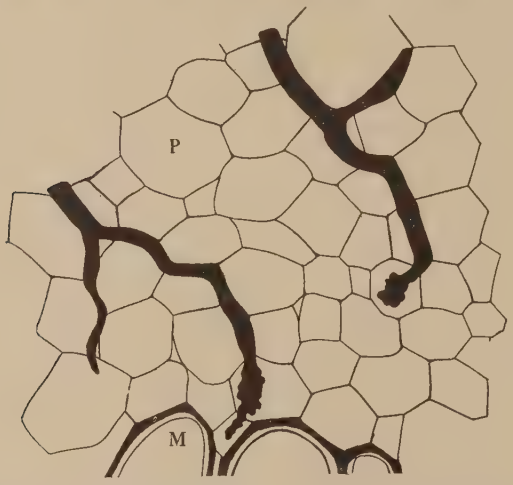

Fig. 2. Transverse section of a young bean stem showing branching stylet tracks of *Aphis rumicis* in the phloem and the sucking out of the cell contents. P = phloem; M = xylem. × 540.

branching tracks so frequently seen in the phloem region, indicate that this tissue is particularly sought after. Text-fig. 2 shows a simple case of branching in the phloem of a young bean stem. However, cells of the epidermis, cortex, pith, mesophyll of the leaf and xylem vessels may be tapped for nourishment, especially when the host is heavily infested or in poor condition. In Text-fig. 3, which is a section near the tip of a young bean stem, the stylet track after passing through the cortex is branching out in the interfascicular or medullary ray tissue, two branches passing to the phloem and the other two to the pith cells. Plate II, fig. 3 shows a single track direct to the phloem and about to enter a companion

cell. This insect was apparently killed soon after the phloem was reached and hence branches of the track had not yet been formed. Plate I, fig. 1 shows the actual piercing organ passing through the phloem and entering a companion cell.

An interesting case is seen in the transverse section of *Rumex* flower stem infested with *Aphis rumicis*, Plate I, fig. 4. Three stylet tracks pass into the xylem vessels, into which a quantity of saliva has been secreted. Again in Fig. 2 on the same plate the actual piercing organ is seen forcing its way through the thick pericycle sheath of a *Rumex* stem into the phloem.

I have found epidermal cells filled with saliva and the contents of the cells destroyed, the stylet track eventually ending in the first or second row of cortical cells. This probably represents cases in which the aphids were killed before the piercing organ had penetrated deeper into the tissues.

Cortical cells are frequently found plasmolysed and sucked out. This was especially noted with *Macrosiphum* on the rose (Text-fig. 4 *a*) and *Myzus* on the peach. This sucking out may be produced by a deliberate piercing of the cell (Text-fig. 4 *a*) or by the destruction of the cell wall by intercellular penetration and secretion of saliva.

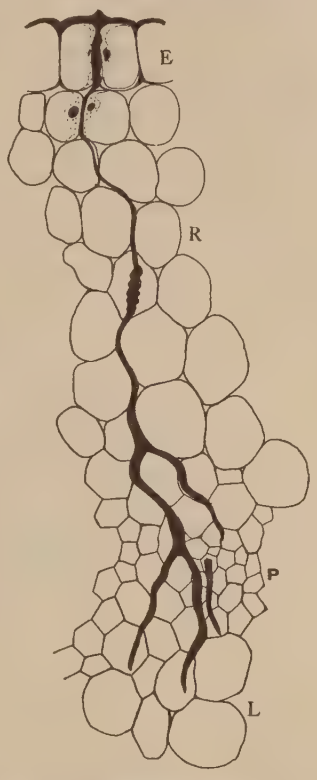

Fig. 3. Transverse section of a young bean stem showing branching of the stylet tracks of *Aphis rumicis*. The epidermis and two rows of cortical cells are shown in detail in Plate I, fig. 3. E = epidermis; R = cortex; P = phloem; L = pith. × 225.

It is seen that the piercing organ frequently follows the line of the cell wall. Apart from the value of this in assisting penetration as explained in a previous paragraph, it is economical, in so far that a greater number of cells are tapped than if the line of penetration was always directly intracellular.

Normally, *Aphis rumicis* lives quietly in close colonies on young parts of the plant or beneath the leaves. During a heavy infestation however, the turgor of the cells is considerably reduced owing to the action of the saliva and the withdrawal of cell sap by the aphids. The supply of sap therefore is greatly affected, a wilting of the shoot occurs and the insects

in their search after food become restless, wander over the plant and tap the cells of many different tissues for nourishment. While normally the colonies below the leaves are situated on the mid rib or veins (the phloem being on the under side of the leaf), in heavy infestations they often wander over the leaf surface and puncture the spongy mesophyll tissue.

Two young bean plants were infested in a localised area, each with two apterous individuals of *Aphis rumicis*. After 24 hours this part of the plant was fixed with the aphids *in situ*. Only four stylet tracks were found in the sections and they passed to the phloem tissue.

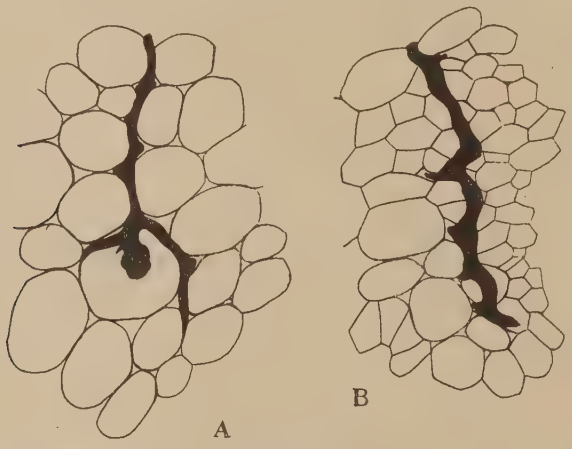

Fig. 4. Transverse section of stem of Rose showing:
A. intercellular passage of the stylet track of *Macrosiphum rosarum* in the cortex and sucking out of a cortical cell;
B. showing stylet track in the phloem. × 500.

Some species of aphids do, however, exhibit differences in their method of obtaining nourishment from the plant. Petri for example has shown that *Phylloxera* on the roots of the vine takes its nourishment from the cortical cells and does not reach the vascular bundles. It would appear that the differences in anatomical structure occurring in plants may affect the relative accessibility of the tissues rich in sap, especially the vascular bundles. The distribution of sclerenchyma, for example, may be important. Further there is the question of the differences which may occur in different species of aphids, as for example the length of the stylets. It must be remembered that it is the young forms which have to be especially considered.

The varying constitution and concentration of the cell sap of different

plants and of the same plants at different periods of growth, is doubtless an important factor influencing the development of aphids. Speaking generally, the cell sap consists of a number of dissolved substances, which are the products of the metabolism of the plant, being usually acid owing to the presence of certain organic acids. The following substances may be present in cell sap (Strassburger's *Text-Book*, 1921).

(*a*) Inorganic salts, especially nitrates, sulphates and phosphates.

(*b*) Soluble carbohydrates, such as cane sugar, malt sugar, glucose and grape sugar.

(*c*) Amides, especially asparagin and albuminous substances. Alkaloids and glucosides are also often present. Tannin is frequently found in the cortical cells and some authors consider that on this account these cells are largely protected from aphid attacks. The presence of tannin associated with the stylet track has already been discussed.

From the anatomical and cytological considerations discussed in this paper, it is evident that the saliva can digest the celluloses of the cell wall, more especially the middle lamella which is largely composed of calcium pectate. Frequently one sees the stylet track passing intercellularly through the thick walls of the stout supporting tissue located at the angles of the stem in *Rumex* and *Vicia faba*. This tube-like track shows distinctly the dissolving action of the saliva.

The quality and consequent food value of the sap varies in the different tissues. The cell sap of the cortical parenchyma is moderately rich, but the phloem elements which serve to transport (sieve tubes) the rich products of assimilation throughout the plant, or as temporary storage cells (phloem parenchyma), have naturally a richer concentration of nutritive substances.

The intercellular spaces of the cortex are largely filled with water and dissolved gases. In the case of trees, the parenchyma cells of the cortex and pith become filled towards the end of summer with reserve substances, chiefly starch. When the buds burst in spring the action of the diastase ferments in the plant change these insoluble reserves into sugars, which are translocated to the buds.

Normally the supply of sap in the phloem is plentiful and it is rich in albuminoid substances, carbohydrates and inorganic salts, especially phosphates.

The xylem vessels (tracheae) are essentially vessels for conveying the watery solutions of organic and inorganic substances up the plant. Dixon and Atkins(9, 10) have shown however, that monosaccharides and disaccharides are also found in the sap of the tracheae in all the trees these

authors examined, the concentration of these substances being greater in spring than in autumn.

We see therefore, that with *Aphis rumicis* the phloem elements of the vascular bundles, are the chief source of the food supply, although other cells of the plant may also be tapped for nourishment. The rapid development and reproduction of aphids on young growing tissue is evidently associated with the quality and rich concentration of the cell sap. These observations also indicate, that the varying susceptibility of different plants to aphid attacks, which the writer has recently investigated, is associated with the constitution and concentration of the cell sap. Investigation of the cell sap of plants at different periods of growth and under varied manurial and cultural conditions is necessary. While great stress has been laid on the value of soluble carbohydrates as food for aphids, one must not overlook the fact that proteids are equally important. The amount of proteid matter compared with carbohydrates in cell sap is relatively small. In young growing tissue on the other hand, the sap is rich in substances of the amino-acid type.

VIII. The Excretion of Honey Dew.

Finally one may briefly refer to the honey dew of aphids, the production of which is closely associated with the digestive processes.

If with the aid of a lens, one watches *Aphis rumicis* feeding on a young succulent plant, it will be seen that the individuals frequently raise the posterior end of the abdomen and eject a large drop of liquid from the anus. This is the so-called honey dew, which often forms an adhesive layer on plants infested with aphids. In many cases this excretion becomes covered with a black fungoid growth, which grows rapidly on the medium. On *Euonymus europaeus*, *Aphis rumicis* excretes similar drops from the anus, but these dry and leave a white deposit on the leaves and the fungoid growth does not develop on them. This substance responds to the Fehling's solution test, indicating the presence of reducing sugars.

Büsgen records that fungi do not develop on the excrement of *Aphis padi* on Prunus nor of *Aphis euonymi* on *Euonymus*.

On some young *Euonymus europaeus* bushes which were heavily infected for experimental purposes, the writer observed the black fungus did develop on many leaves, but normally the fungoid growth does not develop on the excrement of *Aphis rumicis* on *Euonymus*.

The stomachs of apterous viviparous females fed on *Euonymus* and beans respectively, were examined five hours after feeding, on April 14th,

in normal saline solution. In every case, of those fed upon *Euonymus*, the gut was found full of a white crystalline substance composed of long needle-shaped crystals. In the case of the aphids on beans, the stomach was smaller and filled with fluid and numerous oil globules, and there were only a few isolated crystals. Some a. v. ♀♀ from *Euonymus* were examined 30 hours after feeding and the large crystalline mass was still present in the stomach.

It would appear therefore, that the composition of the cell sap affects the nature of the excrement or honey dew. The medium thus produced may not in some cases be suitable for the growth of the fungus. Further the relationship between ants and certain species of aphids, the former being attracted by the honey dew, probably depends largely on the specific composition of that substance.

No recent analysis of the chemical constitution of honey dew has been carried out, but Boussingault(4) and later Brandes(5), showed that in the cases investigated there was a high percentage of sugars. Boussingault showed that there was an increase in dextrine and invert sugars, indicating a breaking up of the cane sugar of the plant juices by the digestive processes of the aphids. Aphids appear to feed almost continuously and large quantities of soluble carbohydrates are taken up in the cell sap. As these insects do not possess Malpighian tubules, the waste products, including surplus sugars, are passed out through the anus.

From the observations described above, it is clear that the cell sap is an important factor in relation to the physiology of aphids, a closer investigation of which, in relation to the composition of honey dew, may throw more light on the biology and physiology of these insects.

IX. Summary.

(1) The food of aphids is the cell sap of plants, which they obtain by penetrating the tissues by means of a flexible, chitinous, piercing organ, which is composed of the maxillary stylets and the mandibles.

(2) The maxillary stylets are closely apposed, thus forming two canals, which extend to the extremity of the compound stylet thus formed. The dorsal canal is the suction canal along which the plant juices are conducted into the pharynx. The ventral canal is the ejector salivary canal down which the saliva is pumped into the plant.

(3) The penetration of the piercing organ is brought about by the retraction of the labium and the forward prolongation of the "forehead." The labium at its distal end grips the piercing organ in a pincer-like manner.

(4) With *Aphis rumicis* the piercing organ passes intercellularly through the cortex, only occasionally passing through individual cells. Eventually it reaches the vascular bundles.

(5) The saliva is able to dissolve a passage for the piercing organ through the walls of the cells. It is also able to convert starch into sugar.

(6) A well marked sheath, the stylet sheath, is formed round the piercing organ when it is in the plant tissues, its wall being composed of substances produced by the reaction of the saliva on the cell sap. It probably consists of callose and insoluble calcium pectate together with a deposition of tannin.

(7) The saliva causes plasmolysis of the cells and disorganisation of the cell contents.

(8) With *Aphis rumicis* on beans, the phloem elements of the vascular bundles are the chief source of the food supply, although other cells of the plant, such as the cortex and mesophyll of the leaf may be tapped for nourishment, especially when the plant is heavily infested. Frequently in *Rumex*, xylem vessels are tapped for food.

(9) The presence of a thick cuticle may prevent young aphids from piercing into the tissues and inhibit a general infestation of the plant.

(10) The sucking out process is usually intracellular, although intercellular suction sometimes occurs in the loose cortical tissue.

(11) In the case of *Macrosiphum* on the rose, occasional cells react under the influence of the saliva, resulting in a cellulose thickening of the cell wall of affected cells.

(12) The varying constitution and concentration of cell sap in different plants is an important consideration in respect to the biology and physiology of aphids.

(13) The more favourable development and reproduction of aphids on certain plants is probably associated with the nature of the cell sap, and an investigation of the cell sap of plants under varying cultural conditions and manurial treatment appears to be necessary.

(14) The composition of the excrement of aphids, or honey dew, depends upon the species of plant and aphid concerned, and is in close relationship with the composition of the cell sap of the plant and the digestive processes of the aphid.

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XI. EXPLANATION OF PLATES I AND II

PLATE I

Drawings showing the effects produced by *Aphis rumicis* on *Rumex* and *Vicia faba*. The staining methods employed are described in the text.

Fig. 1. Transverse section of *Rumex* stem, showing the piercing organ passing through the pericycle (*I*) into the phloem (*P*). Note the stylet sheath (*S*) (coloured red) surrounding the piercing organ. ×540.

Fig. 2. T.S. *Rumex* flower stem showing the piercing organ passing through the epidermis (*E*), cortex (*R*) and pericycle (*I*) into the phloem. Note the secretion of saliva (coloured red) at the tip of the piercing organ, and the displacement of the pericycle cells by that structure. ×500.

Fig. 3. T.S. of bean stem showing the course of the stylet track (coloured red) and the effect of the saliva on the cells. Note the contents of the stylet sheath forced out on the surface of the epidermis. ×500.

Fig. 4. T.S. of *Rumex* flower stem showing the stylet tracks in the pericycle (*I*) and phloem and the penetration into the xylem vessels. ×540.

PLATE II

Drawings showing the effects produced by *Aphis rumicis* on *Vicia faba*, and *Rumex*; *Myzus cerasi* on the peach and *Macrosiphum rosarum* on the rose.

Fig. 1. T.S. of leaf petiole of peach showing destruction of the cells and the secretion of saliva in the phloem region. × 500.

Fig. 2. T.S. of young bean stem showing intercellular course of the stylet track. Note the irregular globules of saliva in the intercellular spaces. × 500.

Fig. 3. T.S. through bean stem showing intracellular stylet track in the phloem, leading into a companion cell. × 540.

Fig. 4. Radial section through a rose stem showing the piercing organ of *Macrosiphum rosarum* passing through the tissues to the phloem and entering a sieve tube. Note the stylet sheath (coloured red) surrounding the stylets.

Fig. 5. T.S. of *Rumex* stem showing stylet track passing along the middle lamella of the cell walls in the pericycle. × 540.

REFERENCE LETTERING.

S = stylet sheath. *P* = phloem. *I* = pericycle. *R* = cortex. *E* = epidermis. *M* = xylem. *O* = piercing organ. *G* = starch grains. *T* = pith cells. *X* = maxillary stylets. *D* = mandibles.

(*Received June 12th*, 1922.)

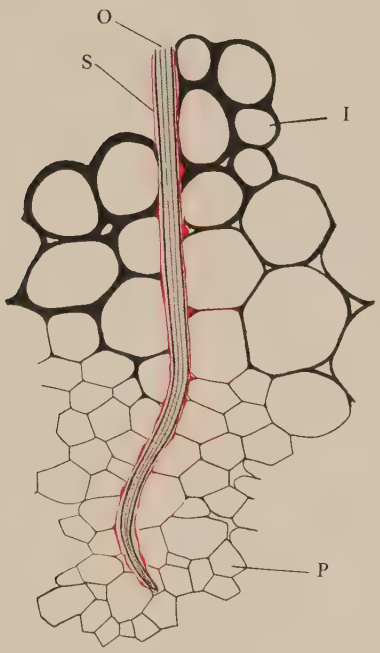

Fig. 1

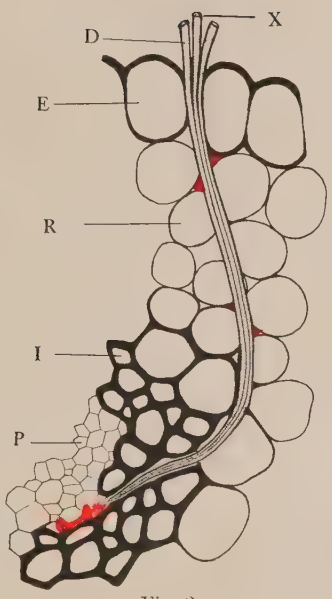

Fig. 2

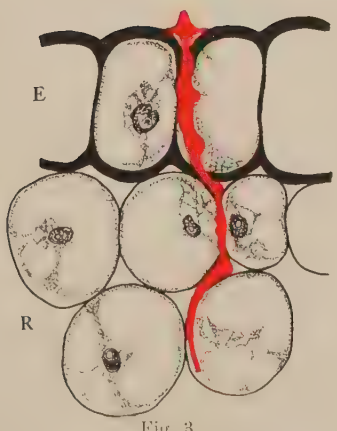

Fig. 3

J. D. del.

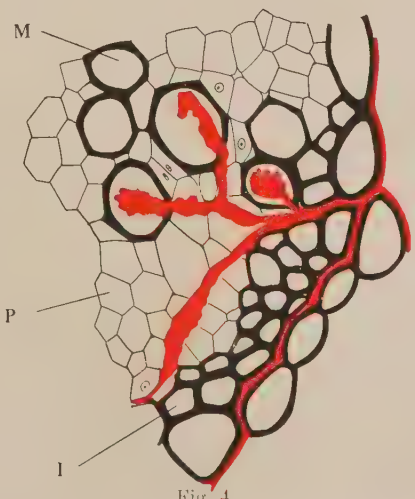

Fig. 4

"VIRUS DISEASES" OF ANIMALS

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The first disease attributed to a "filter-passing virus" was Mosaic disease of the tobacco plant which was described by Iwanowski in 1892 (11). The next, namely, Foot and Mouth disease of cattle was investigated by Loeffler and Frosch (14) and their observations on the virus were recorded in 1898, the same year in which Beijerinck (4) published his rediscovery of the virus of Mosaic disease. Since then, however, the animal viruses must have far outrun those affecting plants in number, since about 48 or 50 are already on record.

The chief points of interest common to virus disease of animals and plants appear to me to be

(1) The nature and properties of the virus.

(2) The means of transmission.

(3) The means which can be adopted for prevention.

The usual synonyms for a "virus disease" have been a disease caused by a filter-passing organism or by an ultra-microscopic virus. In what follows, however, the term "virus disease" is taken to mean an infective disease which can be transmitted experimentally, though it is not due to any known protozoon, spirochaete or ordinary bacterium, but can be transmitted by a liquid containing only very minute particles—smaller than ordinary well-known bacteria. This definition is not very exact, but the boundaries of the group are so ill-defined that it is impossible to cut it off by a sharp line.

The group includes a large number of "viruses" most of which may be classified as filter-passers, but also admits some which will not pass through a Chamberland or even a Berkefeld filter when suitable precautions are taken to prevent the smallest cultivable bacteria such as *B. pyocyaneus* or *B. prodigiosus* from passing. Filtration through a so-called "bacterial" filter is not a very satisfactory criterion of size, since much depends on the rate and duration of the filtration and on the pressure used, as well as on the readiness with which the virus is adsorbed on to the surfaces of the filter. The consistency of the liquid in

which the virus is contained is also of great importance, for with a thick viscous colloidal suspension or undiluted blood serum, the pores of the filter rapidly become narrowed and blocked. Moreover, the smallest members of a young culture of some bacteria are probably as small as the larger individuals of some of the coarser viruses, *e.g. Rickettsia*, the presumptive causal organism of Typhus and Trench fevers, and the minute bodies seen in cultures and described as the micro-organisms of Influenza and Fowl diphtheria, which are believed by their sponsors to be filterable.

To examine critically the filterability of a virus it must be compared with a known emulsion of small bacteria. Both should be tested with the same filter under as nearly as possible the same conditions of time, pressure and temperature and in a liquid as nearly as possible of the same composition or consistency.

If, on the other hand, the visibility of the active agents in a virus is to be taken as a criterion for classification, the difficulties are not much less. Almost all the diseases which I am considering are caused by viruses whose microscopic appearances are either quite unknown or in dispute; in a number of instances the morphology of the organism has been described but either its pathogenicity has not been tested by animal inoculation, or culture and inoculation, or else claims to have done this with success are not generally accepted.

Since both the above criteria—filterability and visibility—depend to a large extent on the size of the particles composing the virus it is necessary to consider what order of size these methods are concerned with. *The size of particles*, which will pass a Chamberland filter (*F*) or a good Berkefeld is generally stated at about 0·2 micron in diameter (0·0002 mm.) (Rosenthal). This approximates very closely to the size of particles which can be clearly resolved with the high power of the microscope, which is put at 0·15–0·2 micron. It has been calculated(8) that the smallest living organism cannot be much less than 2 micro-microns (2/1000 micron) and would probably be not less than 5/1000 micron in diameter. This calculation is based on the size of molecules large enough to contain the constituents of living protoplasm (proteins and salts). That is to say, a living organism can be conceived, of which the diameter is 1/40 of that of the smallest clearly visible particle. This is a disputable calculation except as a minimum. The ultra-microscope is capable of revealing particles only 4–5 micro-micron (4/1000 to 5/1000 micron) *i.e.* 1/50th of the smallest clearly visible particle and about the same size as the smallest conceivable living organism. The ultra-micro-

scope, however, gives no certain information with regard to size or shape and, therefore, does not enable minute micro-organisms to be distinguished from unorganised particles. A further property viz. diffusibility has been attributed to some viruses, *e.g.* of Rabies in glycerine (17) and of Mosaic disease in agar jelly (4). Since the former of these viruses is not very readily filterable through a Berkefeld, it seems unlikely that in its usual form the virus is truly diffusible, but this question awaits further work.

It is seen then, that it is not at all an easy matter to define the properties of the pathogenic viruses, and classification of them is still more difficult and really a matter of rough lists which may be used for comparing them in studying their properties. A good classification is not possible at present as so few of their properties are clearly ascertainable and viruses which agree in one particular, *e.g.* resistance to glycerine, like Vaccinia, Poliomyelitis and Rabies, differ in other respects, *e.g.* readiness with which they pass through a bacterial filter. And those which pass through a porcelain filter, *e.g.* the viruses of Foot and Mouth disease, Horse sickness, Poliomyelitis and Encephalitis lethargica differ in regard to their resistance to drying, exposure to phenol, glycerine etc.

It must be remembered that the diseases in this group are thrown together partly because so little is known about their causation and from time to time so-called filter passers are found to possess distinctive and clearly recognisable pathogenic microbes which are not at any rate in all conditions so extremely minute. For instance, the serum of patients containing the virus of Yellow fever was stated to pass a Chamberland filter *F* in an undiluted state and a finer Chamberland *B* when diluted by Rosenau, Parker etc., whereas, more recently Noguchi and others appear to have shown that true Yellow fever is due to a slender spirochaete (*Leptospira icteroides*) of about 0·2 micron diameter but very much longer.

The diseases under consideration may be roughly divided into four classes:

Class I. The largest; visible, not filterable, not cultivated.
,, II. Intermediate; probably visible, filterable, cultivated.
,, III. The least in size and the most inscrutable; filterable, not cultivated (*a*) visible in animal tissues, (*b*) not visible.
,, IV. Filterable, very resistant.

It must be understood that these attributes are assigned to the different viruses on a balance of evidence, and that such a term as "visible" only means that there is good reason to believe they can be or have been clearly seen under a high power.

Table I.

Classification.

I	II	III		IV
Visible Not filterable Not cultivated (Rickettsia)	Visible Filterable Cultivated	Filterable Not cultivated Visible? (strongyloplasmata)	Not visible	Resistant ? Visible Not cultivated
Typhus	Pleuro-pneumonia	Rabies	Foot and mouth	Infectious
Trench fever	of cattle	Sheep-pox	Rinderpest	anaemia of
Rocky mountain	Avian diphtheria	Fowl-pox	Swine fever	horses
spotted fever	Fowl-plague?	Fowl-plague?	Distemper	Fowl-pox
	Influenza	Vaccinia	Dengue	Fowl-sarcoma
	Poliomyelitis	Variola	Fowl-leukaemia	Horse-sickness
		Molluscum	Mumps	
		contagiosum	Measles	
		Trachoma		
		Scarlatina		

Class I. The virus is probably not filterable through a Berkefeld which will hold back ordinary small bacteria, but it has been considered filterable at some time by a competent observer. Forms of about 0·2–0·3 micron in diameter which are believed to be the pathogenic micro-organisms can be seen and identified in stained films. This group includes Rocky Mountain spotted fever, Typhus fever, and Trench fever. Claims have been made that the forms known as *Rickettsia* and seen in the virus of Typhus and Trench fever have been cultivated on artificial media from patients and lice infected with Typhus but this is not yet generally accepted.

Class II. Viruses which are intermediate in their known properties, and are related to certain diseases, *e.g.* Pleuro-pneumonia of cattle, Poliomyelitis or Infantile paralysis, Influenza and Avian diphtheria. The virus of each of these it is claimed has been filtered and cultivated, the pathogenic organisms seen in stained films of the cultures and the diseases reproduced with subcultures far removed from the original material. The minute organisms described in Poliomyelitis, Avian diphtheria and Influenza appear to closely resemble the strongyloplasmata of Lipschütz(13) which are included in Class III.

Class III. Comprises a large number of diseases which are transmissible by a virus which is believed to pass a Berkefeld or Chamberland, but with regard to some of them there is difference of opinion or rather in experience of different workers, and there is no doubt that the ease with which an active filtrate can be obtained is very variable among the members of the Class. In section (*a*) of Class III very small

bodies named strongyloplasmata by Lipschütz(13) have been seen in the tissues, inside cells and also in the virulent discharges and have been credited by Prowazek(16) and Lipschütz(13) with being the causes of the diseases. These minute bodies are about 0·15-0·2 micron in diameter, are rounded or dumbbell-shaped as a rule and occur in very large numbers. In speaking of strongyloplasmata Lipschütz says(13) "In the affections under consideration (Vaccinia, Variola, Sheep-pox, Molluscum contagiosum, Trachoma, Fowl-pox, etc.) the morphology of the causal organisms is so similar and uniform that it must be looked upon as a remarkable freak of nature that micro-organisms which can scarcely be distinguished should have such extraordinarily diverse biological properties" (Kolle and Wassermann, 2nd Ed. VIII, p. 365). Prowazek called these minute forms elementary bodies and considered them a stage in the life history of the pathological micro-organism which was represented at other stages by larger bodies which he called initial bodies and much larger latent forms which he saw in the inclusion bodies in cells such as the Negri bodies of Rabies, and the Trachoma inclusions. The elementary bodies are described as usually intracellular, but may be intranuclear, *e.g.* in *Virus myxomatosus* and *Gelbsucht*, or extracellular (Trachoma) or in cell-free fluid as in Pleuro-pneumonia of cattle.

The following 10 or 11 diseases which it is claimed are associated with the Chlamydozoa or Strongyloplasmata and in which the pathological changes are attributed to these bodies are

Fowl-plague (Prowazek).
Fowl-epithelioma or Fowl-pox (Borrel).
Sheep-pox (Borrel and Paschen).
Vaccinia (Prowazek and Paschen).
Molluscum contagiosum (Lipschütz).
Variola (Prowazek).
Trachoma (Halberstadter and Prowazek).
Scarlatina (Paschen, Bernhardt and Cantacuzene).
Virus myxomatosus of rabbits (Beaurepaire-Arago).
Alastrim.
Gelbsucht (Prowazek).

The appearance of these bodies (strongyloplasmata) very much resembles some forms found in cultures of poliomyelitis virus (Flexner and Noguchi), common Cold (Foster), Influenza (15), Fowl diphtheria (6) etc. so that the claim to have cultivated these latter has encouraged work with a view to the cultivation by similar methods of the strongyloplasmata. There has been and still is a great deal of hesitation about accepting the strongyloplasmata as pathogenic organisms and this is extended in a less degree towards the very minute somewhat similar forms seen in cultures and for the same reasons.

This is due largely to the difficulty of recognising such very small objects which are often stained with difficulty and of distinguishing those associated with a given disease from similar bodies found in other diseases and also from mere precipitates and globules in emulsions of colloidal substances which occur in smears of animal tissues and in certain culture media especially those containing serum or blood after a few days' incubation. Most of these stain red with Giemsa's stain and resemble strongyloplasmata very closely. It has recently been suggested by Woodcock (20) that these are all the result of red blood cell or nuclear disintegration.

The recognition, identification and differentiation of these very small forms under a high power of the microscope is then very difficult and at first appears impossible. Experience, however, in searching for the very slightly larger and more definite Rickettsia-bodies of Typhus and Trench fevers has taught one that possibly the difficulty may not be insuperable with practice, if the range of size and shape and the arrangement of the elements are specially considered, and if a large number of the forms are viewed, so that too much importance is not attached to the individual forms. This is particularly important since in all or almost all these very small bodies in viruses and even in the case of *Rickettsia* the prevailing shapes are round, dumbbell or diplococcal, and similar shapes are taken by precipitated or finely divided protein or other colloidal matter.

When, however, a large collection of these forms is examined it may be seen that as in the cases of *Rickettsia quintana*, the round and very short forms are almost invariably present in greatly preponderating numbers, whereas *R. Prowazeki* takes on longer and even thread-like forms and shows a much greater range of variation in size, especially under certain conditions, and it is usually intracellular. Moreover, *R. quintana* stains a purplish colour with Giemsa and *R. Prowazeki* stains a much redder colour. In the same way more variation in form and much larger individuals are seen in films of *R. melophagi* though the commonest forms are rounded and stain purple like *R. quintana*. *R. lectularii* is intracellular in some stages and also forms long threads; it stains red with Giemsa.

While it is perhaps necessary to preserve a rather sceptical attitude towards the strongyloplasmata as causes of disease, still their possible importance cannot be hastily set aside especially as evidence is forthcoming that very similar forms can be cultivated in artificial media and reproduce the disease from which the material started.

The position seems to be that in some cases films showing strongylo-

plasmata or culture forms resembling them are described and demonstrated by one observer whereas others are unable to distinguish the bodies in question from similar objects found in uninoculated culture media, blood, etc.

I confess that so far this latter has been my position with regard to preparations from common Cold, Influenza, Vaccinia, Foot and Mouth, and some others which I have had the opportunity of seeing.

For instance, in attempts by the writer to culture the virus of Trench fever very large numbers of round and dumbbell-shaped minute bodies were found in films of the culture tubes after incubation. These usually stained red with Giemsa. They were in some instances extremely numerous and had a certain resemblance to *Rickettsia* and at the time were not seen in preparations from control culture tubes of culture media. The appearances were so striking that it was thought justifiable to try and reproduce the disease by inoculation, but the attempt failed and later many forms were found in the control tubes which we were unable to distinguish from those in the supposed cultures.

In the same way "lymph" from a "foot and mouth" vesicle on several occasions appeared full of minute strongyloplasmata. The suspicion was heightened by the belief that the lymph contained a very concentrated virus. These forms were not, however, always found in virulent lymph and they, moreover, resembled granules seen in uninfected tissue material and in attempted but inactive cultures. The picture seen in the virulent lumph very closely resembled the plates given by V. Betegh(5) of forms believed by him to constitute the virus (see also Huntemuller's observations on F. and M. lymph).

The conditions necessary for a presumption of causal relationship of a given body or bodies, seen under the microscope but not cultivated, to a disease must include quite definite evidence that the disease and the microscopic bodies are associated in a high proportion of cases examined and that the forms seen are not found under similar conditions apart from the disease. That the bodies are so characteristic as not to be liable to confusion with other forms seen in other diseases or under similar circumstances is a necessary part of the evidence.

If these criteria are fulfilled a close association of the microscopic bodies and the disease is proved and a presumption of causal relation is established.

In practice, the degree of probability for this connection depends very much on the certainty with which the disease in experimental animals and the microscopic forms can be identified, the number of cases

and controls examined and the number of cases in which association occurs. In many of the investigations into virus diseases the observations have been very few. The best evidence of the causal relationship of a microscopic form to a disease is obtained by culturing the forms on artificial media through a number of generations and then reproducing the disease in an animal in a clearly recognisable form. Evidence of this kind has been produced in the case of Pleuro-pneumonia of cattle and Poliomyelitis.

In the case of the Influenza virus which under the name of *Bacterium pneumosintes* has been recently cultivated by Olitsky and Gates (15) and in a somewhat similar way by Gordon (9) the disease produced in animals is not so like the original disease in man as to be certainly the same. This is very likely in part due to the want of a suitable experimental animal. One of the most serious obstacles to proving the existence of activity of a virus in culture is the high degree of natural infectiousness of the virus diseases, such as Influenza, Foot and Mouth, Distemper, etc., rendering the isolation of control animals extremely difficult.

Another line of criticism which has to be met is that although the virus may be present and increase in cultures still the *visible forms may not be the true virus* but are formed by the virus or are only incidentally associated with it. Even when the case is a much easier one, *i.e.* when the visible forms are undoubted bacteria, it may be only possible to separate them from the virus by filtration and they may be constantly associated with the virus in diseased animals, although the filterable virus is the primary pathogenic agent and the bacterium is only a secondary invader. This is the case in Swine fever in which the *B. suipestifer* is very frequently present and produces a secondary infection and was therefore at one time accepted as the only cause of the disease on account of its supposed constant presence in disease and its undoubted pathogenicity. The filterable virus of Poliomyelitis (Flexner and Noguchi (7)) and the associated streptococcus are another puzzling instance of associated virus and bacterial infection. Also in Influenza the pneumococci and streptococci very frequently caused secondary infections and it is disputed whether the disease is due to the *B. influenza* or to a filter-passer with the *B. influenza* as a superadded infection. If an ultra-microscopic filterable virus is constantly associated with forms resembling stronglyoplasmata the bacteriologist who attempts to prove the identity or diverse nature of the visible forms and virus needs a stout heart and a long life.

GENERAL CHARACTERS OF VIRUSES.

It seemed at first when filter-passing viruses were discovered that they must have some intangible, indefinable consistency necessitating a new and strange conception of contagion. This is expressed in Beijerinck's phrase *Contagium vivum fluidum*. There has been a tendency to credit the filter-passing viruses as a whole with greater resistance to chemical and physical agents than most bacteria. The tests have as a rule been made with virus contained in the blood, secretions, excretions or tissues, in which it is found in disease. This fact must be taken into account if comparison is made with cultures of bacteria in broth or emulsions of cultures in salt solution, and, moreover, the very high concentration in which some of these viruses must occur has to be reckoned with. It has been stated for instance on high authority, that one millionth of a c.c. of blood etc. from a bird with Fowl-plague or one hundred-thousandth c.c. of the contents of a Foot-and-mouth disease vesicle will transmit the disease.

Few comparative tests of resistance and viability appear to have been made on filter-passing viruses and bacteria as far as possible under similar conditions.

Table II.

Resistance of Virus.

Disease	Glycerine	Drying	Heat	Carbolic
Vaccinia	203 days at 10° C. +	220 days +	60° C. 20 mins. –	...
Rabies	10 months +	4–15 days –	50° C. 1 hr. –	1 % 2–3 hrs. –
Fowl-plague	270 days +	20 days at 22° C. +	Liver 62° C. 30 mins. – / Brain 65°–68° C. 4 hrs. +	...
Pleuro-pneumonia	+	...	58° C. –	...
Poliomyelitis	5 months +	20 days +	56° C. –	1 %
Fowl-pox	170 days +	1½ years +	60° C. dry 3 hrs. +	1 % –
Horse-sickness	...	–	45° C. +	3 % +
Swine fever	...	3 days +	60° C.	...
Rinderpest	8 days –	...	58° C. –	1 % –
"Foot and mouth"	–	...	50° C. –	1 % –
Sheep-pox	12 days –	...	...	...
Typhus	–	Blood –	55° C. –	...
Trench fever	–	Blood – / Louse faeces +	55° C. –	...
Infectious anaemia of horses	...	7 months +	58° C. –	...

+ = persistence of virulence. – = loss of virulence.

Though the characteristics as regards resistance are by no means uniform among filter-passers causing animal diseases, it does not appear that they are very different from those obtaining among bacteria. Thus

most viruses are destroyed at 55 to 60° C. Drying is destructive of some and not of others; glycerin, chloroform, ether and carbolic as well as solutions of bile and saponin are rapidly fatal to some viruses but not to all. It must be conceded that certain viruses, *e.g.* Vaccinia, appear to be far more resistant to glycerin, chloroform and ether than are most non-sporing bacteria. The three last mentioned agents do cause a deterioration or reduction in the virulence or perhaps numbers of the individual organisms in the virus, but the reduction in the number of the non-sporing bacteria seems to be much more rapid, perhaps partly because their number is vastly smaller.

The different effect of drying according to the concentration of the virus (the number of its elements) is probably illustrated by the different effect of drying the virus of Typhus or Trench fever in the blood of a patient and in the excreta of lice. The excreta are far more virulent and contain enormous numbers of Rickettsia-bodies so that less than 1 mg. will cause the disease in a guinea-pig, whereas 2 c.c. of blood is usually required. The louse excreta resist moderate drying whereas blood when once dry appears to have lost its efficacy. There is no other reason to suppose that a different form of virus is present in blood and lice. Associated with the view that the filterable viruses were of a different nature from bacteria or protozoa on account of their resistance the idea was entertained that many of them were very much smaller than the smallest bacterium and were perhaps only visible with the ultra-microscope. The tendency at the present day is to think that in many cases the virus is identical with very minute visible particles. This is encouraged by recent apparent successes in culture, and at any rate appears to be the fashionable view.

No virus of an animal disease appears to be so resistant to heat in a moist condition as are the juices of plants infected with Mosaic disease (70–80° C.) nor will they survive treatment with alcohol as is recorded of the Mosaic virus.

The virus of Bird-pox or Avian epithelioma is amongst the most resistant to drying, heat (60° C. for a short period), and glycerin; the virus of Fowl-plague in brain tissue is stated by Prowazek to resist 65 to 68° C. for four hours as well as desiccation over KOH at 22° C. for 20 days. The viruses of the Infectious Anaemia of horses, Fowl-plague, and Horse sickness are said to remain active amongst decomposing dung for many days. Rabies virus has been found to be still active after exposure to 5 per cent. carbolic for 24 hours. The filterable virus of Fowl-sarcoma in its resistance to drying for long periods resembles these.

The remarkable properties of some of these unusually resistant viruses have suggested that an *unorganised ferment or enzyme* might in some cases act as a virus. The question has been debated since Iwanowski discovered in 1892(11) and Beijerinck(4) rediscovered and discussed the nature of the filterable virus of Mosaic disease. The suggestion that the virus of an infective disease might be an enzyme has usually been rejected as *a priori* untenable since an enzyme cannot be supposed to reproduce itself. Beijerinck called the virus of Mosaic disease a *contagium vivum fluidum*, but appreciating the difficulties of this suggestion he apparently had an alternative or accessory view that the virus was not strictly speaking living on its own account for he proposed that it might enter the cells of the infected plant and become in such a way united with the contents of the cells that when the latter divided the virus might be taken along and divide also with them. This I think implies that something like an enzyme is so tacked on to the living substance of the cell that the cell protoplasm becomes modified and then grows and divides while retaining its new additions which then wholly or in part can be shed or excreted as an independent enzyme.

This is a bold hypothesis but something like it has recently been advocated in a rather different form by Bordet to explain the reproduction or multiplication of d'Herelle's so-called Bacteriophage. It seems possible that the phenomenon of Twort and d'Herelle may throw light on the nature of some of the filterable viruses of infective disease of animals, and it may be best to briefly recall the facts and the explanations which have been put forward.

The phenomenon, as is well known, consists, at any rate as far as concerns our present purpose, in the fact that has often been verified by many observers that when a Berkefeld filtrate containing the active principle has once been obtained it can be multiplied indefinitely by adding a few drops of the filtrate to a tube or flask full of a young culture of susceptible bacteria, *e.g. B. dysenteriae* (Shiga). These latter are dissolved and every drop of the contents of the tube or flask after filtration is found to have become as active, *i.e.* to contain as potent and concentrated a solution as the original drops introduced. This implies indefinite multiplication of the lytic agent, but does not necessarily mean that the "bacteriophage" reproduces itself as a living organism. The active liquid besides passing through a bacterial filter has been found to resist a temperature of 75° C. without destruction.

It is d'Herelle's opinion that the "bacteriophage" is a living microbe and the facts observed by him and others certainly appear to afford

considerable analogy with the filterable viruses of some diseases of higher animals and plants. Almost all other workers beside d'Herelle dispute his conclusions for two reasons.

(1) The liquid containing the lytic agent resists high temperatures, 75° C., and also chloroform and other antibacterial substances.

(2) The phenomena, though recently presented in a new form by Twort and d'Herelle resemble very closely those which have long been known under the name of *autolysis* and it seems probable that the Bacteriophage will be found to be explained by the action of autolytic ferments, *e.g.* such as that derived from the meningococcus and described by Flexner in 1907.

The two hypotheses proposed to account for the phenomenon without postulating a living virus may be briefly stated as follows.

I. Bordet suggests that the phenomena are due to a soluble substance which diffuses into the bacteria and then participates in their division and multiplication and becomes in fact an hereditary factor; the hypothetical substance being thus both *contagious* and *hereditary*. This appears to be very like the hypothesis of Beijerinck.

II. The alternative and more generally accepted view is that the so-called "bacteriophage" is really of the nature of a lytic enzyme which so affects the bacteria in a culture that they break up and in so doing liberate large quantities of autolytic ferment which in turn act like the original lytic substance. This view though not without its difficulties appeals strongly to the present writer.

If an autolytic enzyme originating from a bacterium and able to destroy living organisms of the same species is admitted as the explanation of the d'Herelle phenomenon, then it seems no longer possible to deny *a priori* that an unorganised ferment acting in a similar way on the cells of the animal body might destroy them and in so doing induce the liberation of a fresh supply of a similar ferment. The ferment would then be multiplied or reproduced by the cells of the body though strictly speaking it could not reproduce itself in the sense that a living organism does. The difficulty in conceiving how an infectious disease could be due to a ferment would then resolve itself into the difficulty in understanding how the hypothetical enzyme could be conveyed from one host to another. This does not seem an insuperable obstacle since the ferment might be transmitted in the same way as living organisms by dust, droplets, etc. Such an hypothesis as the existence of an enzyme-virus to explain certain filter-passing infectious diseases is perhaps not necessary and much further work on the more resistant viruses would be needed

before the existence of a living pathogenic microbe could be definitely excluded.

A very short reference must be made to other points of resemblance between the diseases of animals and plants of the kind now under discussion. As such instances of resemblance may be mentioned:

(1) Carriers of disease in the sense of recovered or unrecognised cases of disease which are infective and act as sources and stores of virus from which infection spreads. Such carriers are recognised in Foot and Mouth disease, Trench fever, Influenza, Poliomyelitis, etc.

(2) Insect vectors, *e.g.* biting flies (horse-sickness), mosquitos (Dengue fever), lice (Typhus and Trench fevers), ticks (R.M. Spotted fever). The larva of the kedani mite or harvest-bug (Trombicula) conveys Flood fever or Tsutsugamushi. In most of these diseases, if not in all, a period of incubation in the insect is necessary. In the same way I see that Mosaic disease is believed to be carried by aphides.

(3) Another point of resemblance is that special cells are said to be attacked and appear to constitute the sites of multiplication of the virus.

It has been often observed that many of the animal viruses appear to have a special affinity for certain organs or cells of the body. Lipschütz divides the virus diseases into two main groups.

Group A. Acute general infections comprising 18 diseases.

,, B. Containing 21 localised diseases, of which 3 attack the epidermis, *e.g.* Molluscum contagiosum, Trachoma; 6 the skin, dermotropic, *e.g.* Variola, Foot-and-Mouth; 6 neurotropic, *e.g.* Rabies and Poliomyelitis; 2 haemotropic, *e.g.* Fowl-leukaemia; 4 organotropic, *e.g.* Pleuro-pneumonia and Mumps.

In several of the latter groups Prowazek, Lipschütz and others have described strongyloplasmata or larger bodies in the special cells. *Rickettsia prowazeki* of typhus lives and multiplies in the intestinal cells of the body louse and according to Wolbach and Todd also in the endothelial cells of the small vessels of mammals infected with typhus.

It is, therefore, of interest that Iwanowski (1902) recorded the occurrence of very minute bacteria filling certain cells in Mosaic disease; I do not know, however, to what extent this has been confirmed.

(4) Prevention depends on the same principles in plants and animals except as regards means taken to produce immunity in individuals.

Prevention consists in (i) finding and selecting strains resistant to disease, which has occasionally proved of value in animals but is probably far more important in plants; (ii) isolation or destruction of cases of

disease; (iii) raising the resistance of individuals by feeding and improved hygiene, fresh air etc.; (iv) isolation or destruction of carriers of the virus, when the carriers are not so numerous as to render this impracticable; between and at the commencement of epidemics this may be effective; (v) destruction or avoidance of insects or other vectors.

Conclusions.

1. The so-called "viruses," with very few exceptions are not very different in their properties from a bacterial *materies morbi*.

2. Apparent differences are partly accounted for by the extreme concentration of some samples of virus and the difficulty of comparing the resistance of virus and bacteria under comparable conditions.

3. There is very strong evidence that very small micro-organisms are present in very large numbers in various cells, organs and secretions during some of the so-called virus diseases and that they can be cultivated, obtained in pure culture and, after several passages, reproduce the disease.

4. Some viruses pass through a fine Chamberland filter, but with a considerable loss of strength or virulence. It does not follow that the forms which are seen are identical and of the same size with those which pass through a filter.

5. The difficulty of establishing the causal connection between microscopic bodies of 0·2–0·3 micron and a disease is largely due to difficulties of culture, but the recognition by staining properties and morphology of such minute bodies and the differentiation of those belonging to different diseases from each other and from precipitates and other non-living colloidal particles is also at present an extremely hazardous performance.

6. The analogy with diseases such as Rocky Mountain fever, Typhus and Trench fevers which are associated with definite bodies (Rickettsia) which can be differentiated morphologically suggests that similar differentiation in the case of some particles associated with other diseases, may in time be achieved. The evidence regarding Influenza and some other virus diseases has been strengthened lately but can hardly yet be regarded as conclusive.

7. That an unorganised ferment may cause infective disease does not seem to be theoretically impossible on *a priori* grounds, especially if the phenomena of the lytic substance of Twort and the bacteriophage of d'Herelle are considered as analogous cases.

8. The better known and most thoroughly investigated virus diseases of animals do not seem to need any hypothesis other than that of a

minute micro-organism not unlike a very minute *bacterium* to account for their characteristics and those of their viruses as far as these are known.

9. A few viruses, *e.g.* those of Fowl sarcoma, Horse pernicious anaemia, and Fowl-pox have characters attributed to them which seem to belong rather to an enzyme than a living micro-organism. The alleged diffusibility of Mosaic disease and rabies viruses also suggests a soluble virus.

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N.B. The greater part of the bibliography of the subject will be found in the article by Lipschütz and the handbook of v. Prowazek which are referred to above.

COMMON SCAB OF POTATOES[1]

PART II.

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(With Plates III and IV.)

INTRODUCTION.

An account of some experiments on Remedial Measures for Common Scab has been given by the writer in a recent Report (8). It was there shown that the disease may be inhibited by green-manuring and that, where it is possible to apply a sufficient quantity of green organic matter and to work this into a very intimate mixture with the soil, scab may be entirely prevented. One photograph illustrating this result (Pl. III, Fig. 1) is reproduced from the report. In the experiments described, the green manuring was carried out, for the most part, by the addition of grass cuttings, but this treatment, though simple to apply in the case of a garden or allotment, is obviously impracticable for potato cultivation on a farm. Here, the process must necessarily consist of ploughing in a green crop. The first section of this paper describes some trials of the green-manuring treatment carried out in this way, and gives an account of some further experiments in which hay and spent hops were tried as substitutes for green crops.

The second section deals with the action of liming on scab and the counteraction of its effect by green-manuring. Much attention has been given of late years to the relation of the soil reaction with the incidence of scab and the action of lime on scab must obviously yield valuable evidence in any discussion of this subject. In the third section, an attempt is made to show that the deductions previously drawn from such evidence are fallacious and the various theories put forward to account for the occurrence of scab and its cure by green-manuring are considered.

[1] A grant in aid of publication has been made for this communication.

SECTION I. FURTHER EXPERIMENTS ON GREEN-MANURING AND ON THE USE OF SUBSTITUTES FOR GREEN CROPS.

These were carried out with the object of ascertaining whether the practice of green-manuring, as it obtains in ordinary farm practice, would suffice of itself to inhibit scab, and also whether such substitutes as old hay and spent hops would exert a similar effect.

Green-manuring.

Exp. 1. A plot 22 ft. × 6 ft. was divided into two parts. On one half, three successive crops of mustard were grown and dug in during the summer of 1919 and these were followed by a crop of rye which was dug in in the following spring. Potatoes were then planted on the whole plot, artificial manures only being applied.

The amount of scab on the fallowed half of the plot was unfortunately rather little but the green-manured half yielded a clean crop.

Exp. 2. This was carried out in a garden near Bradford which had been devoted to the growing of a number of potato varieties in 1919. The whole crop was then so badly scabbed that the potatoes were scarcely recognisable. In December 1919, rye was sown thickly over a large strip 57 ft. × 9 ft. across the garden, and the winter being mild, a good crop 10 ins. high was produced by the following May. This was cut with a scythe. The stubble was then dug in, trenches made for planting the potatoes and the green crop strewn in the trenches, little by little, as they were being filled in. For the sake of comparison, another strip was treated with grass at the rate of 15 tons per acre strewn in the trenches as in the case of the rye. This amount of grass had been shown by previous experiments to be an effective dressing for scab. A third strip was planted as a control. No farmyard manure was applied to any of the plots but all were given artificials at the same rate.

The results showed a fair amount of scab on the untreated plot, though this was considerably less than in the previous year. Both the rye and grass plots however gave crops with 90 per cent. clean tubers and no difference between the two plots in this respect could be observed.

Exp. 3. This consisted of a trial of green-manuring on a farm scale. A green crop of rye and vetches 8 to 9 ins. high was ploughed in in the spring of 1920. Within a week of ploughing the land was harrowed and ridged up, most of the green stuff falling into the furrows. Dung was added in the usual way, and the potatoes were then planted. The result of the experiment was, however, negative since both the green manured-part and the control strip which had been left gave clean crops. It is of interest in showing that it is possible on a farming scale to plant potatoes at a very short interval after a green crop has been ploughed in. In this way the decay of the vegetable matter proceeds at the same time as the formation of the new crop, a condition which appears to be essential to the success of the treatment.

The three experiments, all of which were carried out in 1920, suffer from the fact that the amount of scab produced in that year was considerably less than usual, but the results are sufficient to indicate that green-manuring, as carried out by the ploughing in of a growing crop, may be very effective in reducing scab. The writer has since received confirmation of this statement from a well-known firm of seedsmen in this country, who have carried out the treatment with great success, and

also from Mr E. Gram of the Experimental Plant Pathology Station, Lyngsby, Denmark, where Lupins were used as the green crop.

Hay and Spent Hops as substitutes for Green-manure.

Exps. 4, 5. The experiments in which these materials were tested were carried out in 1921, which was probably the worst year for common scab on record.

No old hay was available and seeds hay from the previous year's crop was used. This was applied to two small plots at the rates of 2½ tons and 5 tons per acre respectively. Spent hops, in the wet state in which it comes from the brewery, was applied to two other plots at the rates of 5 tons and 10 tons per acre respectively. In each case a control plot was planted. All the plots received artificials but no farmyard manure. The results were as follows:

Treatment (rate per acre)	Description of crop
Seeds hay (2½ tons)	Badly scabbed
„ „ (5 tons)	Moderately scabbed
Untreated plot (1)	Badly „
Spent hops (5 tons)	Moderately „
„ „ (10 tons)	Very slightly „
Untreated plot (2)	Very badly „

It thus appears that seeds hay is of little use as a substitute for green manure and this is possibly due to its stiff coarse nature, on account of which the attack of soil organisms on it is necessarily slow. It is possible that meadow hay being softer in texture would have given better results.

In the case of spent hops, the heavier dressing gave very good results which would certainly justify further trials being made with this otherwise apparently worthless product.

Section II. The effect of lime on scab.

The literature on common scab abounds with experiments on liming but the results of these are so conflicting that little can be learnt from them. Similarly, potato growers, in some districts, assert that lime has no effect on scab, whilst others, in different districts, or on soils of a different type, regard liming as the surest way of producing a scabby crop.

There is now little doubt that this apparently inconsistent action of lime is closely connected with the reaction and type of soil to which it is applied. In the experiments which follow, either the initial hydrogen-ion exponent or the lime requirement of the soil or measurements of both data were taken in all cases. The type of soil in Exp. 6 was a very light sandy loam; in Exps. 7, 8, 10, and 11 it was a fairly light silty loam, and in Exp. 9 a similar but somewhat heavier loam.

Exp. 6. This was carried out on a farm near Selby. The principal crop was potatoes and these yielded as much as 16 tons per acre of fine clean tubers on which scab had never been seen. In 1916, however, the farmer complained of the poorness

of his rotation crops, in particular of barley and seeds hay, and this was no matter for surprise, when it was observed that the acid indicating weed, spurrey, grew in abundance on the land. A liming experiment was planned and carried out on the field in question by Dr J. A. Hanley, Lecturer in Agricultural Chemistry at this University, to whom I am indebted for the following details. The lime-requirement[1] of the soil was found to be 21 cwts. per acre. Five plots, each one acre in area, were marked out and treated as follows:

No. of plot	Dressing of lime or chalk per acre[2]	
1	Ground chalk (undried)	2 tons
2	„ „ „	$5\frac{3}{4}$ tons
3	Control	
4	Ground quicklime	1 ton
5	„ chalk (dried)	2 tons

In addition, and as a result of the good effect of these dressings on the crops grown from 1916 to 1920, the farmer applied ground chalk at the rate of 5 tons per acre to a further considerable area of the same field. In 1921, the crop was again potatoes and two varieties "Majestic" and "Ally" were grown. At lifting time these were examined by Dr Hanley and the writer, who arrived independently at the same conclusions regarding the amount of scab on the respective plots. These observations, together with the hydrogen-ion exponents of the soils of plots 2, 3 and 4 which were determined on May 19th, 1921, are given in the table below.

No. of plot	*p*H	Degree of scab
1	—	Moderate
2	6·6	Very bad
3	5·0	Practically none. Tubers showing one or two scabs could be found but were not noticeable to a casual observer
4	5·2	Slight
5	—	Rather bad

The "Majestic" crop was perhaps slightly less scabbed than the "Ally," but the relative amount of scab on the different plots was the same in both cases.

On the portion of the field which had been chalked by the farmer the crop was as badly scabbed as on plot 2.

Photographs of the produce of the "Majestic" crop on plots 2 and 3 are shown on Pl. III, Fig. 2.

Exp. 7. This was laid down in the first place as a manurial experiment (which included some liming trials) on pasture land at the University Experimental Farm, Garforth. In 1917, the field was ploughed up and has since carried arable crops, that of 1921 being potatoes. Particulars of the experiment are given in the Guide to the Farm experiments (14), and it is only proposed to state here the details as they concerned the liming of the plots and its effect on the potato crop.

[1] For the sake of clearness, the lime-requirements given in this paper have been expressed throughout in terms of weight of lime per acre. The figures so given, however, are merely calculated from the percentage of calcium carbonate as determined by the Hutchinson and MacLennan method, and must not be regarded as any exact measure of the amount of lime necessary to bring the soil to a neutral reaction. In practice a quantity of lime considerably in excess of that indicated would probably be required for this purpose.

[2] This chalk contained about 15 % moisture.

Each plot was divided into two parts, a north and a south half.

In 1898, the north half of plot 14 was dressed with quicklime at the rate of 3 tons per acre and the south half at the rate of 6 tons per acre. In addition, in 1911, the north end of all the plots was given ground chalk at the rate of 5 tons per acre. The action of this liming was clearly visible on the soil in 1921, four years after the grass had been ploughed in. On the unlimed portions, the ploughed-in turf lay in undecomposed lumps on the surface after harrowing, whilst, on the limed soil, no evidence of the original turf remained. The potatoes planted were "Great Scot." Observations of the amount of scab on the respective crops were made at lifting time and determinations of the hydrogen-ion exponents for two of the soils—one heavily limed and the other untreated—were made. These data are given in the following table.

Plot		Treatment, rate per acre			*p*H	Degree of scab
14	South	Quicklime	6 tons	in 1898	—	Bad
14	North	Quicklime and ground chalk	3 ,, 5 ,,	1898 1911	6·6	Very bad—tubers scarcely distinguishable from the soil
Other plots,	North	Ground chalk,	5 ,,	1911	—	Bad
,,	South	No lime or chalk			4·4	Very little scab—the tubers were spotted to some extent and some showed mature scabs

A photograph of the crops from plot 14 North and from the unlimed plots is shown on Pl. III, Fig. 3.

Exp. 8. This was carried out on a field (No. 112 *S*) at the University Farm in 1915. The lime-requirement of the soil was practically nil. Four plots, each consisting of one row 78 ft. long, were treated as follows:

Plot	Lime applied per acre
1	Untreated
2	5 tons
3	10 tons
4	Untreated

The lime, which was partially slaked, was applied in the rows on top of the dung; a little soil was raked down to cover it and the potatoes then planted.

The crops when examined were found to be all slightly scabbed but no difference whatever could be seen in the relative amount of scab on the four plots.

Exp. 9. This was carried out on a small plot at the University Farm in 1920. The lime requirement of the soil was 9 cwts. per acre, *i.e.* the soil was practically neutral. In this case, it was decided to make a test of magnesian as well as of carboniferous lime, since the former was said to possess a peculiar "burning" effect on root crops. The plot was divided into three parts, which were treated as follows:

Plot	Lime applied per acre
1	Magnesian, 5 tons
2	Untreated
3	Carboniferous, 5 tons

In both cases the lime was partially slaked. It was distributed over the surface of the soil and dug in. Dung was applied in the trenches at the rate of 20 tons per acre and the variety of potato planted was "Great Scot."

All the crops were found to be quite free from scab.

Summary of Experiments 6–9.

The soils on which these experiments were carried out may be divided into two groups according to their reaction before liming. Those of Exps. 6 and 7 were decidedly acid, whilst those of Exps. 8 and 9 were neutral or very nearly so. In the former cases, the application of lime was followed by scab, whilst in the latter, this had no effect. Many similar cases might be quoted and there is little doubt that many of the conflicting statements regarding the results of liming might be reconciled if the initial reaction of the soils treated was known. Superficially, these experiments might be taken to afford full confirmation of Gillespie and Hurst's(2) soil reaction theory for scab, but in the next section, it will be shown that they may be more satisfactorily explained on other grounds. There is no doubt, however, that, on light potato soils, the soil reaction, as measured by its lime-requirement or by its hydrogen-ion concentration may be an extremely useful guide in predicting the action of lime on scab.

The following experiments show that where scab appears on a soil after liming this effect may be counteracted by green manuring.

Exp. 10. This was carried out in 1919 at the University Farm. Two adjacent plots, Nos. IV and V, were chosen on light silty soil. The lime-requirements of the plots were found to be 37 and 38 cwts. per acre respectively. One half of each was given an application of lime at the rate of 50 cwts. per acre, and two days later plot IV was given a dressing of grass cuttings at the rate of 18 tons per acre which was forked in. No farmyard manure was given to either plot, but each received a standard dressing of artificials. Both plots were then planted with "British Queen" potatoes. The results were as follows:

Plot		Appearance of crop
V. Limed half		Scabbed
Unlimed half		Clean
IV. Limed half Unlimed half	} green-manured	Clean

A photograph of the crops from plot IV is shown on Pl. IV, Fig. 4.

In the case of plot IV, a few tubers lying at one edge of the plot, where they had escaped the "grass" treatment, showed scab and one of these is included in the photograph on the right hand side. Otherwise, there was no difference in the crops from the limed and unlimed parts of this plot and both showed the clean glossy skins so typical of the green-manurial treatment.

Exp. 11. This was similar to Exp. 10 excepting that a much smaller dressing of grass was used—not more than 2 to 3 tons per acre—on the green-manured plot. The results were almost identical, excepting that the crop treated with lime and grass, although quite free from obvious scabs, showed numerous pin head spots. These were not investigated for want of time, but, in view of the examination of such spots described in Part I(15) of this work, there is little doubt that many of them were very

young scabs. This is of interest, since it shows that where the supply of green-manure is too limited or becomes exhausted the potatoes subsequently may be attacked by the scabbing organisms.

A photograph of the spotted tubers from this plot is shown on Pl. IV, Fig. 5.

These experiments prove therefore that where lime exerts a tendency towards the production of scab, this may be entirely counteracted by green-manuring. The significance of this result in its relation to the theories put forward to account for scab is considered in the next section.

Section III. An examination of the theories advanced to account for the occurrence of scab and its prevention by green-manuring.

1. *The soil reaction theory.*

This theory doubtless owes its origin to the fact that scab occurs only to a very slight extent in distinctly acid soils. Gillespie and Hurst (2) have now placed this observation on a scientific footing by measurements of the hydrogen-ion concentration of such soils and have further attempted to establish the existence of a close relationship between the hydrogen-ion exponent of *any* soil and the incidence of scab. The conclusions arrived at by these workers are that soils having a hydrogen-ion exponent as low or lower than 5·2 *rarely* produce scab, whilst soils with much higher exponents *generally* produce scab.

We are in agreement with the first part of this statement, although at the same time it should be pointed out that instances of scab in soils of exponents less than 5·2 are not uncommon. Martin (7) has already cited two cases of rather severe scab at exponents of 4·82 and 4·6 respectively, and the present writer mentions the existence of scab in Exp. 7 of this paper, where the exponent was as low as 4·4.

It is, however, with the second part of Gillespie and Hurst's statement that we find ourselves in complete opposition. In the first place we note in the authors' own account of their work that a certain soil "No. 36," having an exponent of 6·22 and falling in the middle of their series of scab-producing soils, yet gave a clean crop of potatoes. This inconsistency was accounted for by supposing that the soil was uncontaminated with scab organisms, since it was "virgin" land. Some years previously, however, Jones and Edson (4) cited a case of scabby potatoes occurring on land which, presumably, had not been in cultivation for 25 years and a part of which had just been cleared of pine. In this case the soil may, of course, have been infected by the use of scabby sets, but it seems much more probable that the Actinomyces capable of producing potato scab are natural inhabitants of the soil.

In any case, however, the argument of "virgin soil" could not be applied in this country, where no kind of control over the planting of scabbed sets has ever been enforced, and where it is impossible that there remain any potato growing soils into which the scab organisms have not at some time or another been artificially introduced. Yet, on thousands of farms clean potato crops are raised and it would be absurd to suppose that in every case the soil was an acid one. Indeed, if we may take such physiological data as the production of a good crop of barley and the absence of acid indicating weeds such as Spurrey, Sheep's Sorrel and Bent on a soil to be a criterion of approximate neutrality, it is an easy matter to recall numbers of such neutral soils, where clean potatoes are regularly grown. In order to verify these observations, it was decided to collect samples of clean potato producing soils from various sources and to determine their hydrogen-ion exponents. The samples were obtained by the courtesy of mycologists, potato growers and gardeners in different parts of England and Scotland and, in each case, the complete or comparative absence of scab is vouched for by the senders. In several cases the land from which the samples were taken was well known to the writer and he is able to confirm the statements regarding absence of scab. The samples were taken to a depth of 6 to 8 inches and at the writer's request, obviously sour soils were avoided.

The values of the hydrogen-ion exponents[1] found are given in the table on p. 78.

Thus, out of 15 soil samples varying from a light sandy loam to heavy clays and including also two peat soils, none showed a hydrogen-ion exponent less than 6·0 whilst the majority gave exponents greater than 7·0. There is no doubt that the number of such neutral or alkaline soils producing clean potatoes could have been added to indefinitely.

These results are obviously a complete contradiction to the conclusions of Gillespie and Hurst who appear to have generalised too quickly from

[1] In estimating the hydrogen-ion exponents of the soils mentioned in this paper the procedure adopted was as follows:

The air dried soil was powdered as far as possible by finger pressure or by gentle pressure with a wooden block. A portion was then put through a 1 mm. sieve. 20 gm. of this was shaken into a specially cleaned flask with 50 c.c. of neutral distilled water and shaken 75 times. After standing for two days the supernatant liquid was filtered through filter paper, samples of which had been found by previous examination to have no effect on the hydrogen-ion concentration.

The earlier estimations were kindly carried out by Mr N. M. Comber, Lecturer in Agricultural Chemistry at this University, who used the colorimetric method of Gillespie (*Soil Science* 1920, IX, 115); the later estimations were made by the writer using the older colorimetric method of Clark and Lubs (*Journ. Bact.* II, Nos. 1, 2, 3). Comparative tests by Mr Comber and myself showed that the two methods gave closely approximating results.

	Source	Type of soil	*p*H	Remarks
1.	Nostell, Yorkshire	Medium loam	7·7	Sent by a head gardener who had never known scab to occur on it. The 1921 crop was examined by the writer and found to be perfectly clean
2.	Doncaster, Yorkshire	Light loam	7·7	No scab known to occur on this soil before 1921 when the crop was very slightly scabbed
3.	Wedmore, Somerset	Medium loam	7·9	From the writer's own garden where scab is unknown
4.	,, ,,	,, ,,	7·6	Scab unknown
5.	,, ,,	,, ,,	7·8	,, ,,
6.	Sand, Somerset	Sandy loam	7·4	Ploughed out grass land in 1917. Subsequent crops free from scab
7.	,, ,,	Heavy loam	7·8	Scab very rare
8.	,, ,,	Heavy clay	7·6	,, ,, ,,
9.	Wellington, Somerset	Sandy loam	7·7	,, unknown
10.	Settle, Yorkshire	Light loam	6·1	These soils overlie limestone. Crops invariably clean
11.	,, ,,	,, ,,	6·0	
12.	Moorland, Somerset	Peat	7·1	Clean crops always produced
13.	Fen district, Cambridgeshire	,,	7·6	,, ,,
14.	Innerswick, Dunbar, Scotland	Red Sandstone	7·1	The clean potatoes grown in this district are well known
15.	,, ,,	,, ,,	7·1	

their work on the Caribou and Washburn soil types. It becomes apparent, therefore, that although a high hydrogen-ion concentration may serve as an indication of the absence of scab in a soil, the converse statement is by no means true.

Some neutral and alkaline soils produce clean crops and others produce scabbed crops, and the reaction of the soil cannot, therefore, be regarded as the determining factor in the incidence of scab.

2. *The preferential food theory.*

This theory was advanced by the writer in an earlier report (8). It was there named the "Decoy" theory, but may here be more accurately described under the name given above. It is based on the established fact that when a sufficient quantity of green vegetable matter is introduced into a scabbing soil, clean potato crops may be grown and a practical point on which some stress has been laid is that the more intimately the soil and green-manure are mixed, the better are the results of the treatment.

Adherents of the soil reaction theory have naturally attempted to explain these results by suggesting that the inhibitory effect of the green-manure was due to a rise in the hydrogen-ion concentration of the soil brought about by its decay. Stephenson (10) has shown, however, that

vegetable matter such as Rape and Soy Beans, when applied in a dry state at the rate of 10 tons per acre actually reduced the hydrogen-ion concentration. A few tests were made by the writer during the summer of 1921 on some green-manured and untreated soils which had been ridged up for potatoes in the ordinary way. The green-manure had been applied as short grass at the rate of 20 tons per acre. Estimations of the hydrogen-ion exponents of the green-manured and untreated soil ridges gave the following results:

Date 1921	*p*H values	
	Green-manured soil	Untreated soil
May 25	4·7	4·5
July 28	4·8	4·4
Aug. 15	4·8	4·4
Oct. 14	4·9	5·0

It should be pointed out that the samples taken were from ridges 6 ft. long and composite samples were not made from the whole of the ridge. This may possibly account for the curious increase in the exponent for the untreated soil between the two last dates on which samples were taken.

In the case of the green-manured soil, however, the figures show a gradual decrease in the hydrogen-ion concentration and thus agree with Stephenson's results.

It appears impossible therefore to account for the effect of green-manuring by the soil reaction theory.

The preferential food theory makes the assumption that *Actinomyces scabies* is primarily a saprophytic organism living on organic remains in the soil and showing a partiality for the more undecomposed parts of vegetable matter. Only when the supply of such food stuff is deficient does it attack the potato tubers.

Shapovalov (9) has already called attention to the saprophytic nature of the organism and its "parasitic possibilities."

The writer has found that sterilised grass cuttings make the finest medium known for rapid growth of strains of *A. scabies* and that sterilised samples of the peat soil from Somerset mentioned on p. 78 also permitted good growth. Photographs of the cultures made on these two media may be seen in the Report (8) mentioned.

On the assumption made, it is easy to understand the scab-inhibiting effect of green-manure and the necessity for a close admixture of the green stuff with the soil, but the theory receives its strongest support from the remarkable way in which it accounts for the established facts concerning scab. These may be considered as follows:

(1) True peat soils irrespective of their hydrogen-ion concentration are comparatively free from scab, and this may now be explained by the fact that, in such soils, the large natural reserve of organic matter serves as a constant food supply to the Actinomyces present. In a recent review of the writer's Decoy theory, Drayton(1) states that he was unable to find any Actinomyces in certain soils, principally peat, or peat and clay, which were of high hydrogen-ion concentration. It will be remarked however that the maximum count given by this author in an examination of 23 soils is less than one million per gram, which is a very low figure. Although, therefore, without any standardised method of making Actinomyces counts, the relative figures of different investigators are of little value, we suspect that Drayton's technique is at fault in finding soils totally devoid of these organisms.

The present writer found as many as 700,000 Actinomyces per gram of the soil mentioned in Exp. 7 which had a hydrogen-ion exponent of 4·4 and on which slight scabbing occurred. The counts for neutral or alkaline soils are however generally much higher and the writer does not question the statement that acidity depresses the Actinomyces flora, and thus reduces the likelihood of scab. This does not in any way vitiate the application of the preferential food theory to peat soils. Thus, the Actinomyces count for the Somerset peat soil mentioned on p. 78 and having a hydrogen-ion exponent of 7·1 was 5,000,000 per gram. Yet, this soil yields perfectly clean crops each year.

(2) Scab is most prevalent and most virulent on light sandy or gravelly soils. The well aerated condition of such soils is peculiarly suited to the development of the *A. scabies* group, and probably of the majority of the genus. Bacterial action is also rapid and thus manure, whether farmyard or vegetable, tends to disappear quickly. The condition thus brought about in such soils is the reverse of that in a peat soil and scabbing ensues. Clean potatoes may sometimes be obtained on such soils by early lifting. It is on land of this type that heavy green-manuring is particularly efficacious.

(3) Lime produces or aggravates scab under certain conditions.

From the experiments described in Section II the following facts stand out:

(*a*) The presence or absence of scab on neutral soils is unchanged by liming.

(*b*) The addition of lime to a distinctly acid soil tends to produce or increase scabbing.

(*c*) This deleterious effect may be counteracted, if heavy dressings of green-manure are applied to the limed soil.

As already pointed out (*a*) and (*b*) if considered alone might be taken as confirmation of the soil reaction theory. A point in connection with liming results is however generally overlooked. According to the soil reaction theory, we have to assume that soils of a certain degree of acidity are void of scab organisms. When, however, such soils are limed, intensely scabby crops may be produced and we may well ask from what source the scab organisms appear in such numbers as to produce this effect.

When next we consider (*a*), (*b*) and (*c*) together, it will be seen that the whole case cannot be explained by the change in soil reaction, since it has already been shown that green-manuring, though exerting only a slight effect on the soil reaction, tends to move it in the same direction as lime.

The facts are however easily accounted for by the Preferential food theory. Lime favours the development of the Actinomyces both by neutralising acidity and by opening the soil. At the same time, it hastens the decay of plant residues in the soil. Thus, on the one hand, the scab organisms are multiplied and, on the other, their natural food supply dwindles. The potatoes are then attacked. If, however, the deficiency in the food supply is made good by the addition of green stuff, the tubers remain free from infection. In the case of (*a*), it is obvious that, where no deficiency of lime exists, a further addition will produce no ill effect. In this case, if the soil is well supplied with vegetable matter in a palatable state for the scab organisms, the crops will be clean, and, if it is not, they will be scabbed.

Some critics of this theory have urged that the green matter introduced, if so favourable a medium for Actinomyces, would be quickly smothered with these organisms and disappear in a very short time. This, however, is not the case, since Actinomycetal action at soil temperature is much less rapid than at the higher temperatures adopted in artificial cultures. In fact, in the green-manuring experiments described, which were carried out on light dry soils, a certain amount of the grass added was invariably seen when the potatoes were lifted.

Others, whilst accepting the idea of the green organic matter as a preferential food have raised the objection that the spores of the Actinomyces will naturally germinate *in situ* and, in some cases, this must be on the potato itself. We are bound to admit that this objection seems very sound. It should, however, be remembered that spore formation in *A. scabies* is very variable. In cultures, it may often be retarded indefinitely by an abundance of food; it is also much checked by an acid reaction of the medium, and by low temperatures. Again, the power

to produce an aerial mycelium with spore formation is often entirely lost by continuous cultivation of the fungus on the same medium. To what extent these various factors affect spore formation of the fungus in a soil highly enriched with vegetable matter, we are not prepared to say. It is an interesting fact, however, that on potato scabs themselves, the aerial mycelium bearing the spores of the fungus is very scanty.

There is lastly the possibility that the latent parasitic power in the scab organisms is developed only under the stress of hunger and thus, spores or fragments of mycelium from a parent mycelium growing luxuriantly on a saprophytic diet are powerless to attack the potato tuber. In 1921, an attempt was made to test this point by inoculating growing potato tubers with a strain of *A. scabies* previously cultured on sterilised grass cuttings for six generations of one week each. In some cases, the skin of the potato was first scratched with a sterile needle and the inoculum rubbed on the scratch with a platinum loop, whilst, in others, drops of the culture emulsion were spotted on to the tubers. In all, 10 tubers were inoculated. Seven of these showed no signs of infection whilst the remaining three showed only two or three very small scabs. Thus, whilst it would be unwise to draw definite conclusions from a single test, the results certainly suggest a loss of parasitic power in the organisms following a generous saprophytic diet.

There does not appear therefore to be any insuperable objection to the Preferential food theory, which may not be removed by an extended knowledge of the morphological and physiological characters of the Actinomyces group.

3. *Soil moisture and soil temperature as factors in scab production.*

Observations extending over a number of years have shown that common scab is much more prevalent and occurs with far greater virulence in dry than in wet seasons. An illustration of this is afforded by recent years. Thus in 1919 and 1921, the growing season was exceptionally dry and the disease very severe, whereas in 1920, which was very wet, little scab appeared.

In this country, dry summers are usually accompanied by heat and wet summers with cold. Thus, in the action of climatic conditions on the disease, two factors, soil temperature and soil moisture, either or both of which may play an important part, must be considered. In America, L. R. Jones and McKinney (5, 6) have attempted to establish a relationship between soil temperature and scab development. They point out that scab is more prevalent in the southern and warmer regions than in the northern and cooler States. They have not so far given any figures

for the temperatures of these soils, but have shown by greenhouse experiments that the optimum temperature for the development of the scab organism in the soil is about 24° C. The geographical evidence in this country is scarcely in support of these observations since scab is, if anything, more general and severe in the north of England than in the south. In fact, the disease appears to have been a serious problem to farmers in Yorkshire long before it was given any attention at all in the south of England.

Following on the hypothesis of Jones and McKinney, a suggestion was made to the writer by Dr Pethybridge of the Royal College of Science and Technical Instruction for Ireland that the beneficial effect of green-manuring for scab might be due to a reduction of temperature in the treated soil such as would result from a mulch. It was decided therefore to carry out experiments to ascertain (1) what reduction of temperature was actually brought about by mulching, (2) the effect, if any, which such a reduction produced on scabbing, and (3) the reduction of temperature, if any, brought about by a green-manuring carried out on the lines of the previous experiments.

An account of the experiments (Nos. 12 and 13) is given below.

Exp. 12. This was carried out in 1921. A plot 22 ft. × 6 ft. was chosen on soil of a scabbing nature. Its lime-requirement was found to be 33 cwts. per acre and a dressing of lime was applied at the rate of 2½ tons per acre. Later, the plot was dunged at the rate of 15 tons per acre and planted with "Great Scot" potatoes. It was then divided into three parts, *A*, *B* and *C*. In order to carry out the mulching as effectively as possible, the potatoes were not earthed up on any part of the plot. When the tops were about 6 ins. above the ground, *A* and *C* were given heavy mulches about 3 ins. thick of grass cuttings and farmyard manure respectively, whilst *B* was left untreated. A thermometer enclosed in lead piping was then inserted to a depth of 6 ins. between two plants in a row in each of the three plots. Eleven readings were taken at intervals throughout the season from June 18 to October 12 and were as follows:

Date 1921	Temperature ° C.		
	A (Grass mulch)	*B* (Untreated)	*C* (Manure mulch)
June 18	16·0	18·5	16·0
„ 25	19·0	21·5	18·5
„ 28	17·0	20·0	18·0
July 6	16·0	17·0	16·0
„ 14	18·0	20·0	19·0
„ 26	18·5	20·0	18·5
Aug. 3 (after rain)	15·0	15·5	15·5
„ 15 „ „	15·0	16·0	15·5
„ 22	17·5	18·0	17·25
„ 27	16·0	16·5	17·0
Oct. 12	14·5	15·0	14·5
Average of readings	16·6	18·0	16·9

From the above it will be seen that the reduction in temperature effected by the grass and dung mulchings was practically the same and that this amounted only to an average of 1·25° C.

A good crop of potatoes was lifted in spite of being grown on the flat and the results as regards scab were as follows:

Part *A* (grass mulched)	Only moderately scabbed.
„ *B*, untreated	Badly scabbed.
„ *C* (dung mulched)	„ „

No difference whatever between *B* and *C* was apparent, so that obviously the reduction of temperature effected on plot *C* had exerted no retarding action on the scabbing and the diminished amount of scab of plot *A* cannot therefore be ascribed to this cause. It may, however, be accounted for by an observation made on lifting the crop, namely, that a certain amount of the fine grass used had partially rotted and become incorporated with the uppermost layer of the soil. Thus, since the crop lay very close to the surface of the soil, a large proportion of the tubers were growing in a mixture of soil and grass such as they would get in a green-manurial treatment carried out in the ordinary way.

Exp. 13. On a second plot, a green-manurial experiment was put down on half of which grass cuttings were applied and forked in at the rate of 20 tons per acre. Thermometers were inserted in one ridge of the "grassed" soil and one ridge of untreated soil adjacent to each other and readings taken at intervals. These were as follows:

Date 1921	Temperature ° C. "Grassed" soil	Untreated soil
July 14	22·0	23·0
„ 25	22·0	22·5
Aug. 3	17·5	18·5
„ 15	19·0	20·5
„ 22	19·25	21·0
„ 27	17·0	18·0
Sept. 28	15·5	16·5
Oct. 12	15·0	16·0
Average reading	18·4	19·5

The average reduction of temperature therefore due to the admixture of the grass is just over 1° C. and in view of the results of Exp. 12, we must conclude that this reduction can have no appreciable effect in inhibiting scab. In any case, it would be scarcely possible to suppose that so small a difference in temperature would retard the growth of the scab organism to more than a very slight degree. The temperatures noted in the last table certainly approach the optimum of 24° C. given by Jones and McKinney(6) for the scab organism and in such a summer as that of 1921 it is more than probable that the high soil temperature would favour the development of these organisms. On the other hand, however, soil temperatures taken in 1914, on a plot which produced a very scabby crop were considerably lower. Thus, 12 readings taken at 11 a.m. at intervals from July 28 to August 17 gave an average temperature of

13·2° C. and a maximum temperature of 14·3° C., whilst 12 readings taken at 1 p.m. at intervals from August 18 to September 2 gave an average temperature of 16·0° C. and a maximum of 18° C. Such temperatures are much the more normal for this part of the country, but they fall considerably below the optimum for *A. scabies*.

Whilst admitting, therefore, that, provided other conditions are favourable to the disease, a high soil temperature will favour its development, we do not think that this factor can exert more than a secondary rôle in the incidence of scab. It appears far more probable that the moisture content of the soil is the seasonal factor of greater importance.

Soil moisture. In the report(8) on Common Scab already referred to, experiments were described which led us to abandon the hypothesis of the moisture content of the soil being the factor directly responsible for the presence or absence of scab on any given soil and the factor which appeared to us to be fundamental in scab control has already been discussed in this section.

Nevertheless, on soils already favourable to scab, the water content appears to exert an indirect but often powerful influence. It should first be pointed out that the climatic conditions affecting scab only do so when they are very pronounced. Thus, a very dry year brings much scab and a very wet year little, but a moderately wet or moderately dry year has little effect one way or the other.

It is well known that dryness means high soil aeration especially of the surface layers of the soil and excessive wetness means that, except on extremely light soils, the pores of the soil will be constantly choked with water and aeration will be poor.

Previous workers on the Actinomyces group have shown that these organisms are very sensitive to the air supply. Waksman(13) states that out of a large number of species isolated from the soil none grew under strictly anaerobic conditions. Similarly, Mr S. Burr, Demonstrator in Agricultural Botany at this University, has found that none of 12 strains of *A. scabies* examined gave any growth under strictly anaerobic conditions. Most of the strains were able to make some growth under partially anaerobic conditions, as, for example, at the bottom of a liquid culture, but this was greatly increased with a further supply of air. It may safely be assumed therefore that the members of the *A. scabies* group are preferential aerobes. They are, moreover, extremely resistant to drought and cultures which were allowed to dry up have been found viable after a period of two years. Many strains, indeed, produce their spores most readily in culture when the medium begins to dry up.

Thus, it would seem that the continuously high aeration of the soil in a dry year is particularly favourable to the development of the scab organisms, and, in the light of this conclusion, it is possible to explain two facts regarding the incidence of scab which seem incapable of explanation in any other way. Thus (1) certain very gravelly or sandy soils are so totally unaffected by climatic conditions that they produce badly scabbed crops in all seasons. An instance of this type of soil may be found in an account of potato scab by Seton and Stewart(12) and the soil there referred to is well known to the writer. Such soils are so porous that they are never clogged with water even in the wettest seasons and the Actinomyces in them do not suffer at any time from lack of air. (2) Clay soils are far less liable to produce scab than lighter soils. Here, again, this partial immunity to scab would seem to be closely associated with the restricted air supply which is at a minimum in a heavy soil. Soil aeration therefore appears to be the dominant factor in the influence exerted by dry and wet seasons on scab.

SUMMARY OF PARTS I AND II.

1. Common Scab consists of a number of types of scab, which vary considerably in general appearance. Of these, the two most marked types have been called "Raised" and "Pitted" respectively. Other types are intermediate between these extremes.

The causal organism in all cases examined belongs to the Actinomyces genus. The different strains isolated exhibit considerable differences in culture but, for the present, they may be placed in a single Actinomyces scabies group.

2. The incidence of scab is closely associated with soil type. It occurs most commonly and with greatest virulence on light, sandy or gravelly soils, especially on those of a "hungry" nature. Conversely, it is rarely found on peat soils.

3. The disease may be inhibited by the application of sufficiently liberal dressings of green-manure to the soil. Spent hops have also proved to be of value in this respect and leaf mould has long been used by gardeners with good results.

4. The action of lime or chalk on scab production depends largely upon the initial reaction of the soil. On neutral soils, it exerts little or no effect. On distinctly acid soils, it tends to produce or aggravate the disease unless the soil contains a large reserve of vegetable organic matter. This effect may be counteracted by green-manuring.

5. The hydrogen-ion concentration of the soil is not the direct factor

of control in the occurrence of scab and this statement is borne out by the following facts:

(*a*) Scab is found to only a slight extent in soils of a high hydrogen-ion concentration, yet, it *may* occur and Actinomyces have been found in large numbers in soils with a *p*H value as low as 4·4.

(*b*) In more nearly neutral soils, there is no relation between the hydrogen-ion exponent of the soil and the occurrence of scab.

Thus, for example, one soil having a *p*H value of 7·0 may yield absolutely clean crops, whilst another with the same exponent may produce very scabby crops. This distinction cannot be explained by supposing the former soil to be virgin and uncontaminated with scab organisms.

(*c*) In the treatment of scab by green-manuring, any change brought about in the soil reaction appears to be towards a decrease rather than an increase in the hydrogen-ion concentration.

6. The established facts concerning the common occurrence of scab on light soils, its comparative absence on peat soils, the action of lime and the cure of the disease by green-manuring may be explained by the Preferential food hypothesis. According to this theory, the scab organisms are primarily saprophytic living on vegetable remains in the soil. They remain so until their natural food supply is exhausted and develop their parasitic tendencies only under the stress of hunger.

7. Scab is more prevalent in dry than in wet seasons. This is no doubt due in some small degree to the higher soil temperature obtaining in dry seasons. The climatic factor of greatest importance, however, would seem to be rainfall which modifies the air content of the soil and thus regulates the development of the scab organisms which are strongly aerobic. The effect is most marked in clay soils where scab is almost entirely inhibited by a wet season.

The writer wishes to express his sincere thanks to the following colleagues: Mr F. T. Bennett, Mr S. Burr, Mr N. M. Comber, Dr J. A. Hanley, Mr J. Manby, Professor Seton and Miss L. Scott.

EXPLANATION OF PLATES III AND IV

Fig. 1. Effect of green-manuring on scab. Left: green-manured. Right: untreated. (Reproduced from *Report* No. 118, University of Leeds and Yorkshire Council for Agricultural Education.)

Fig. 2. The effect of liming on scab. Experiment 6. Left: chalked. Right: untreated.

Fig. 3. The effect of liming on scab. Experiment 7. Left: limed and chalked. Right: untreated.

Fig. 4. The effect of lime counteracted by green-manuring. Experiment 10. Left: green-manured but unlimed. Right: green-manured and limed.

Fig. 5. The effect of lime partially counteracted by green-manuring. Experiment 11. Note the pin point scabs on all the potatoes.

Note. The blocks for the above plates have been kindly lent by the University of Leeds and the Yorkshire Council for Agricultural Education.

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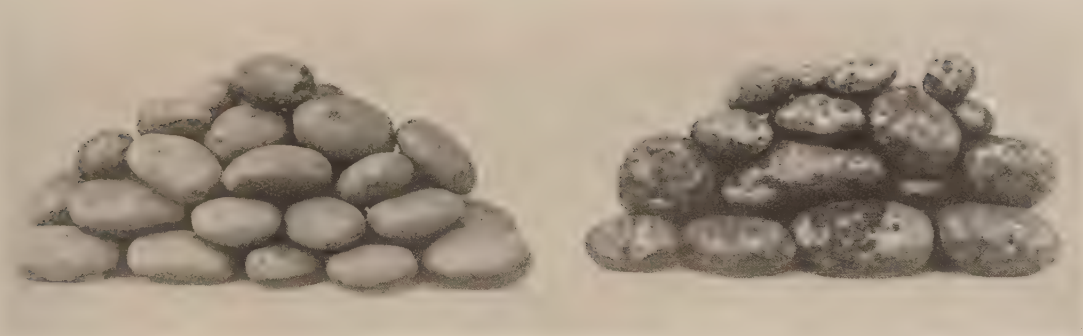
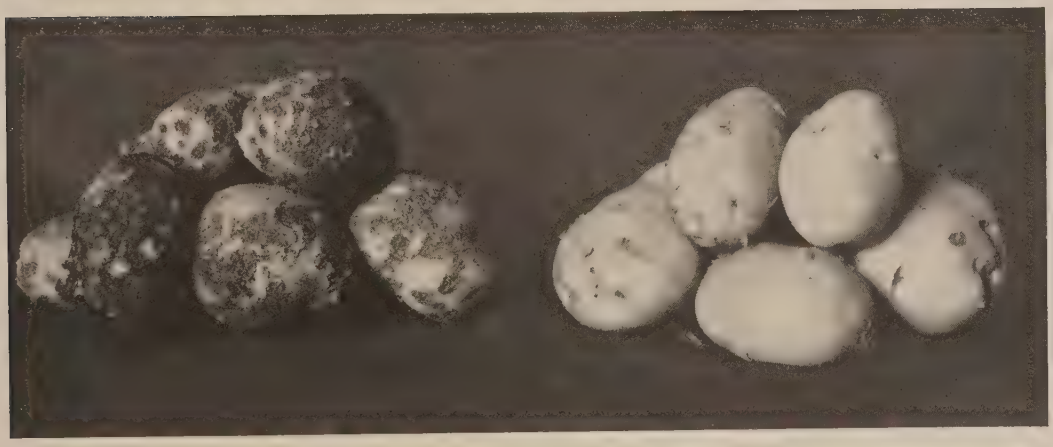


Fig. 1.



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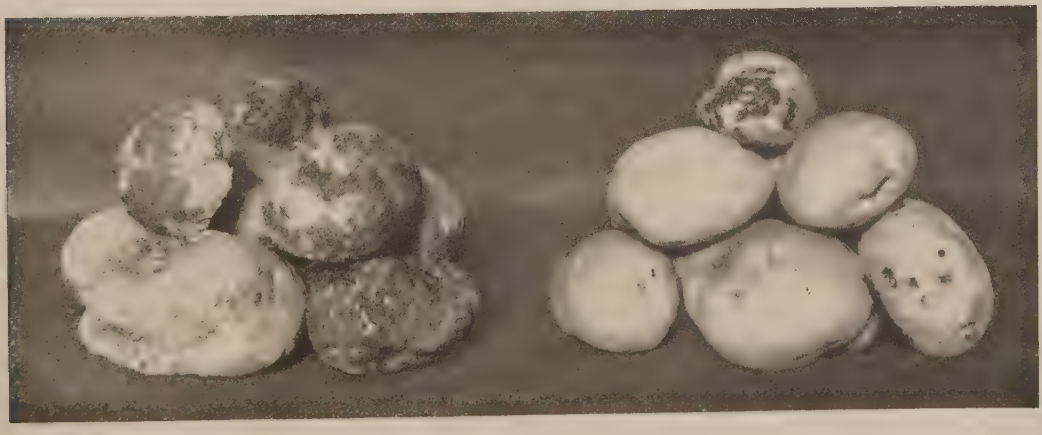

Fig. 3.

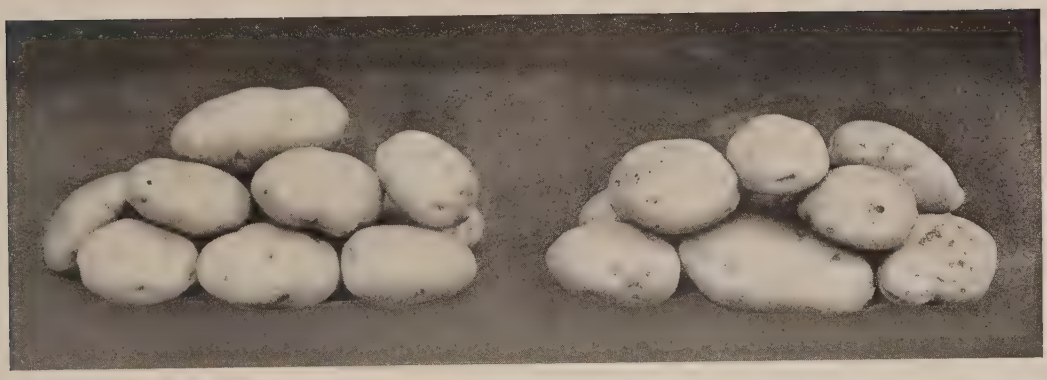

Fig. 4.

Fig. 5.

STUDIES IN BACTERIOSIS. VIII

FURTHER INVESTIGATION OF THE "STRIPE" DISEASE OF TOMATO

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In a previous communication(2) the results of an investigation upon "Stripe" disease of the tomato were described. The isolation of the causal organism, a yellow bacillus, and its resemblance to *B. lathyri*, Manns and Taubenhaus(1) was reported. Owing to the fact that the identity of the organism was not discovered until after the paper had been sent to the press, it was not possible to publish precise proof of identity, namely the cross-inoculability of the "Stripe" organism from the tomato to the sweet pea. The present paper is concerned with the results of a series of cross-inoculation experiments of the "Stripe" organism from the tomato into a number of other plants, chiefly legumes.

Experimental.

Bacillus lathyri was again isolated from typical "Stripe" lesions on tomato stems and tested for pathogenicity by pricking a small piece of a pure culture, under aseptic conditions, into rapidly growing young tomato plants, when the typical lesions of the disease were readily produced. Inoculations with the organism were then performed upon a large variety of plants. This was done in three ways; by seed inoculation, by stab inoculation and by spraying the plants with a suspension of the organism. The plants showed "soft" growth as a result of liberal dressings of a fertiliser containing a high percentage of nitrogen; the best conditions for the disease were thus provided. *Seed inoculation* gave positive results in a limited number of species, but the stab and spray inoculations indicated that a large number of plants are susceptible to the organism.

Stab inoculations. Small pieces of a pure culture were pricked into the base of the stem, under aseptic conditions, and the wound covered

with tinfoil. The external lesions were some time in appearing, usually from four to seven weeks, but on opening the stem with a knife in the region of the point of inoculation, the destruction of the pith could be seen within a week of the inoculation.

Culinary Pea (*Pisum sativum*).

17. iii. 20. Inoculated.
4. v. 20. Small brownish black sunken spots and thin streaks appeared on the stem up to five inches above the stab.
26. v. 20. Lesions showed an increase in size and number and had appeared on the leaves.

Control plants (pricked with a sterile needle) were quite healthy.

Lupin (*Lupinus* sp.).

17. iii. 20. Inoculated.
29. iv. 20. Thin brown streaks on the stem just above the stab.
6. v. 20. Leaves showed many minute sunken black spots.
26. v. 20. Stem showed numerous purple brown streaks and blotches, while the affected leaflets were turning yellow and many had fallen off the petiole.

Control plants were quite healthy.

Red Clover (*Trifolium sativum*).

17. iii. 20. Inoculated.
20. iv. 20. Long light brown sunken streaks appeared on the inner side of the petioles.
26. v. 20. Six petioles per plant showed lesions; the leaflets were covered with small brown spots and patches.

Controls were healthy.

Sainfoin (*Onobrychis sativa*).

17. iii. 20. Inoculated.
19. iv. 20. Thin black sunken streaks appeared on the inner sides of the petioles.
26. v. 20. Many petioles were smothered with lesions, while the leaflets showed black sunken spots.

Control plants were quite healthy.

Potato (*Solanum tuberosum*).

Potato tubers, variety Epicure, were planted in loam in six inch pots and when about four inches high were inoculated.

14. vii. 20. Inoculated.
27. viii. 20. Light brown sunken furrows appeared on the shoots above the point of inoculation.
5. ix. 20. Lesions on the stem were more numerous and larger; some furrows being three centimetres long and three millimetres broad.
10. ix. 20. Inoculated plants had turned yellow in those parts of the stem where the lesions were most pronounced, but there were no lesions upon the leaves.

Controls were quite healthy.

Seed inoculations. Seeds were sterilised in mercuric chloride, washed in sterile water and inoculated by soaking for fifteen minutes in a water suspension of the bacilli. After drying, they were planted in sterilised soil. Seeds treated in a similar manner, but soaked for fifteen minutes in sterile water instead of the bacterial suspension, served as controls. After six weeks, the culinary pea and red clover were the only species to show signs of the disease.

Spray inoculations. For the purpose of this series of experiments the bacterial suspensions were prepared in two ways as follows:

(1) Twenty-five c.c. flasks with 15 c.c. of beef bouillon each were sterilised and inoculated from a beef agar slant culture. After five days' incubation at 22° C. each 15 c.c. were diluted to 500 c.c. by the addition of sterile water at 22° C.

(2) Ten c.c. of sterile distilled water at 22° C. was added to a five days' culture on beef agar and the bacterial mass scraped off by means of a platinum needle. The bacterial suspension was poured from the tube and made up to 500 c.c. by the addition of sterile water. Six plants of each kind were sprayed by means of a small hand spray with suspension (1) and six with suspension (2). Six plants were sprayed with sterile water as controls. Precisely similar results were obtained with each suspension.

Culinary Pea (*Pisum sativum*).

7. vi. 20. Inoculated.
11. vi. 20. Numerous lesions appeared as tiny sunken black spots on leaves, tendrils, pods and stems.
14. vi. 20. The leaves were abundantly spotted; the spots running together to form larger patches and the leaves beginning to wither. The lesions on tendrils and stems showed as numerous blackish brown sunken furrows, four to seven millimetres in length. The pods showed dark brown sunken spots and patches.
20. vi. 20. The plants were smothered in lesions, which in places were two to three millimetres wide. Many leaves were completely withered. The organism was re-isolated from the lesions.

Control plants were quite healthy.

Red Clover (*Trifolium sativum*).

7. vi. 20. Inoculated.
16. vi. 20. Tiny light brown spots appeared on leaflets and petioles.
20. vi. 20. Long light brown sunken furrows on petioles. Leaflets spotted and withering. The organism was re-isolated from typical lesions.

Control plants were quite healthy.

Broad Bean (*Vicia faba*).

7. vi. 20. Inoculated.
16. vi. 20. Tiny dark brown spots showed on the leaflets and stem.
20. vi. 20. Leaflets well spotted with dark brown sunken spots; several withering. Thin purplish brown streaks three to six millimetres in length showed on the stem. The organism was re-isolated from the lesions.

Control plants were healthy.

French Bean (*Phaseolus vulgaris*).

7. vi. 20. Inoculated.
14. vi. 20. Light brown spots on leaves and light brown patches and furrows on stem, petioles and leaf veins.

20. vi. 20. Lesions fairly numerous, but not so far advanced as the other species. The organism was re-isolated with difficulty.

Controls quite clean and unspotted.

Sweet Pea (*Lathyrus odoratus*).

7. vi. 20. Inoculated.
14. vi. 20. Light brown spots on leaves. Tissues yellowing round the spots. Light brown furrows, sunken and splitting, on leaf petioles.
20. vi. 20. Lesions more numerous and intense. Stem, petioles, leaves and flowers all attacked. Organism re-isolated.

Controls healthy.

Lucerne (*Medicago sativa*).

7. vi. 20. Inoculated.
18. vi. 20. Small brown sunken spots on the leaves and similar streaks on the stem.
20 vi. 20. Spots and streaks larger and more numerous. Organism re-isolated.

Controls quite healthy.

Lupin (*Lupinus* sp.).

7. vi. 20. Inoculated.
16. vi. 20. Small sunken black spots on the leaflets and light brown streaks on the stem.
20. vi. 20. Many leaflets covered with lesions. Spots larger and concurrent. Leaflets turning yellow and falling. Stem covered with dark brown lesions, which are splitting longitudinally. Organism re-isolated.

Controls healthy.

Vetch (*Vicia sativa*).

7. vi. 20. Inoculated.
16. vi. 20. Many small light brown to reddish purple spots on leaflets and petioles.
20. vi. 20. Some leaflets almost completely covered with lesions. Leaflets yellowing and withering. Organism re-isolated.

Controls healthy.

Sainfoin (*Onobrychis sativa*).

7. vi. 20. Inoculated.
15. vi. 20. Numerous black spots on leaflets and petioles.
20. vi. 20. Many leaves covered with lesions, turning yellow in places and withering. Petioles covered with short black sunken furrows two to four millimetres in length. Organism re-isolated.

Controls healthy.

Negative results were obtained with *Melilotus*, *Ulex* and *Lotus corniculatus*.

The re-isolations of the organism from diseased plants in the above experiments were grown for five days upon potato-mush-agar and when pricked into young rapidly growing tomato plants readily produced the typical "Stripe" lesions. Their cultural reactions also showed them to be identical with the organism employed in the original inoculations.

It will be seen, therefore, that the organism which causes "Stripe" disease of tomato has a wide range of host plants, a fact which is of great importance to the commercial grower. Many cases are recorded where tomatoes grown in houses built upon pasture, clover leys etc., have been badly attacked by "Stripe" in the first year. This has been in the past a puzzle to the practical man, who naturally expected a clean healthy crop on the new soil. Careful investigation has shown that whenever "Stripe" occurred in the first year, the preceding crop had included legumes. In cases brought to the personal notice of one of us (W. F. B.) the previous crop was clover, lucerne and field peas respectively.

Again those parts of the houses, which have been occupied by hedges in the previous year are frequently to be distinguished by the position of "striped" plants, which mark the spots where vetches and other leguminous weeds grew previously. The whole of the evidence emphasises the necessity for removing all weeds from the vicinity of nurseries. Growers who contemplate building nurseries upon fresh land, would do well to have the land examined by an expert to ascertain if the existing crop is free from "Stripe" disease.

DISEASE TRANSMISSION BY TOMATO SEED.

It has long been suspected that "Stripe" of tomatoes is sometimes transmitted by the seed. While definite scientific proof has not so far been forthcoming, observations on commercial nurseries indicate that this is extremely probable. One case in particular was very suggestive. Until 1920 this nurseryman had grown tomatoes for over 14 years without "Stripe," but this year he bought a little seed to make up a shortage. Plants raised from the bought seed developed "Stripe" and were the only plants out of a considerable number that were so affected. Similar cases have been repeatedly brought to the junior author's notice, and so vital does the problem appear that experiments were set up to investigate it.

Two sets of seed were used; one obtained from a badly diseased fruit and another from an artificially inoculated fruit. The seeds were carefully extracted and dried in 1919 and sown in sterilised soil the following year. Sterilised seed-boxes, pots and water were also used. The plants raised from the naturally infected fruit showed two out of sixteen with "Stripe" and those from the inoculated fruit showed one out of sixteen. Such results are far from being conclusive but they are probably more instructive than at first appears. They were the only plants out of two hundred in the house that developed "Stripe." Microtome sections have

been made of seeds from diseased and inoculated fruits and in some cases the bacilli have been found under the testa. This rather indicates that the bacilli may sometimes be carried in this way, but there is always the possibility that seeds attacked by the organism may be destroyed by them and so prevented from transmitting the disease.

The effect of different manurial treatments on the incidence of the disease.

The yearly observations begun last year in the experimental houses at Cheshunt were continued in 1920. They confirmed those of the previous year, *i.e.* that plants showing hard growth produced by suitable dressings of potash are more resistant to "Stripe" than those showing soft growth resulting from excess of nitrogen unbalanced by suitable amounts of potash.

Variety	Treatment	Total No. of plants	No. of diseased plants	
			1919	1920
Comet	C.A. without potash	120	78	105
,,	Control untreated	120	50	68
,,	C.A. with dung	120	45	50
,,	C.A. without phosphates	120	41	51
,,	C.A.	120	40	41
,,	C.A. without nitrogen	120	34	30
,,	Double C.A.	120	34	34
Kondine Red	C.A. without potash	120	33	96
,, ,,	C.A. with dung	120	33	38
,, ,,	Control untreated	120	30	48
,, ,,	C.A. without phosphates	120	28	31
,, ,,	C.A. without nitrogen	120	19	27
,, ,,	Double C.A.	120	14	31
,, ,,	C.A.	120	13	31

C.A. = complete artificials.

"Streak" disease of the broad bean and culinary pea.

During the early summer of 1920, diseased broad beans and culinary peas were observed growing on an allotment near to a nursery. The soil consisted of scrapings from tomato houses mixed with old cucumber soil. The similarity of the lesions to those obtained in the preceding inoculation experiments with *Bacillus lathyri* suggested that the causal organism was the same. Isolations yielded a yellow bacillus identical with *B. lathyri* which readily attacked the bean and pea and produced "Stripe" lesions when pricked into young tomato plants.

Symptoms.

(1) *Broad bean.* Dark purple brown spots and patches appear on the leaves, petiole and stem. The leaf spots, while small at first, soon enlarge and spread over the lamina, which becomes distorted and withers. Spots and blotches on the stem spread rapidly and become long broad streaks. The pods are disfigured by spots, blotches and streaks and finally become completely smothered with lesions.

(2) *Culinary pea.* The base of the stem first shows typical lesions, which appear as small black sunken spots, blotches and streaks, but the infection soon passes up the stem covering leaves, petioles, tendrils, stem and pods with lesions. The tissues round the lesions gradually turn yellow and then brown. Finally the plants turn yellow, wither and die prematurely. The organism may enter the plant either by means of the root or through the stoma at the base of the stem, which it reaches in water splashed up from the soil. Within the plant the pith is browned and destroyed and in the hollow parts of the stem, the cavities are lined with dead cells.

SUMMARY.

The organism which produces "Stripe" disease of the tomato is shown to be the cause of a number of "Streak" diseases of other plants, namely sweet pea, culinary pea, broad bean, french bean, red clover, lucerne, lupin, vetch, sainfoin and potato.

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THE HEALING OF WOUNDS IN POTATO TUBERS AND THEIR PROPAGATION BY CUT SETS

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(With 3 Text-figures.)

CONTENTS.

Introduction.

The methods by which cut surfaces of potato tubers heal over so that the tissues within are again protected from excessive evaporation and against the entry of pathogenic organisms, have been made the subject of a somewhat extended investigation. The general question as to the methods by which injuries in parenchymatous tissues are repaired, which has been discussed elsewhere (12), will probably receive more elucidation through the intensive study of a few special cases than from a more extended survey of a wider number. The potato tuber provides parenchymatous tissues in a form exceptionally suitable for such investigation, and it is hoped that the special study of this particular case will also have some bearing on the more general question of the resistance of plant tissues to wound injury. At the same time the restriction of the investigation to the potato tuber has enabled a certain amount of attention to be directed to other questions of considerable general interest. Data are given below as to the relative activity of the processes of wound reparation in different varieties of potato. These data may have a certain interest in relation to the behaviour of the varieties, both as regards their suitability for propagation by cut sets and their resistance to

disease, and possibly even as to their general vigour of growth. It is too early to assess the value of these data in this connection but they can at least be placed upon record. Finally the close study made of the natural process of wound healing has thrown some light upon the practice of propagating potatoes by means of cut sets. Some little discussion of this practice is therefore included and an attempt made to define certain conditions which may militate against successful propagation by this method.

It will be necessary first to describe in some detail the method by which cut surfaces in the potato tuber are normally healed. Experimental investigation seems to throw considerable light upon the conditions essential for the operation of the mechanism, and these will be discussed before the question of propagation by sets is dealt with.

The view advanced in an earlier paper(12) will be further developed below, namely that in the process of healing of an exposed parenchymatous tissue, certain processes follow in regular order. First the walls of the cells at the surface of the exposed tissue become covered with a deposit of a fatty nature (suberin). The deposit forms relatively rapidly and as a result of its formation the intercellular spaces of the tissue below the exposed surface are again cut off from the external atmosphere. This process is therefore spoken of as a preliminary "blocking" of the cut surface. An aqueous sap containing solutes collects behind the blocked surface and the result is that the living cells behind the blocked surface commence to divide actively, forming the meristem which, because the new cork layer arises as the result of its activities, is known as the cork phellogen.

I. The "blocking" of the cut surface.

Upon cutting a potato tuber across and leaving the cut surface exposed to air certain changes in the tissue are indicated by the appearance of the cut surface. The exposed surface quickly darkens in colour, the change being due to oxidation products resulting from the action of oxidising enzymes; thus the colour change proceeds more rapidly if the cut surface is exposed to an anaesthetic such as chloroform, but fails to occur if the tissue is exposed to a jet of steam for a few minutes immediately after cutting. Later this superficial black or brown stain is covered by a white, partially crystalline deposit, consisting of inorganic salts, starch, etc. left as the sap upon the cut surface evaporates. If the cut potato is exposed to sunlight or to very dry air, this outer crust of dried matter becomes exceedingly hard and later cracks violently, the cracks opening up avenues into the uninjured tissue behind.

When sections made at right angles to the cut surface are examined under the microscope, another result of the exposure of the surface is revealed. Within a period of some 12 to 36 hours at room temperature the walls of the cells in the close neighbourhood of the cut surface, though not necessarily the outermost cells of all, appear darker owing to a brown deposit upon them. The brown deposit is different from that found within the surface cells as the result of the enzyme action previously mentioned, and is noted only upon the walls. Appel(1) was the first to realise the significance of this layer, which he pointed out blocked the healthy tissues within from the penetration of fungi or bacteria. The time that this deposit takes to form is of considerable importance as, until it is complete, potentially pathogenic organisms may find an entrance to the healthy tissue within.

The Nature of the Blocking Deposit.

This deposit under most conditions (certain exceptional cases will be discussed later (p. 105)), forms a continuous layer along every wall of every cell at a certain depth below the injured surface. Sometimes it extends only over a layer one or two cells deep, in other cases it extends to a depth of seven or eight cells. In either case it is a continuous barrier between the protoplasts, cell walls and intercellular spaces of the healthy tissue within, and the atmosphere outside. It is therefore of the utmost importance to know its nature and the conditions of its formation.

The problem has been discussed previously(12) and the conclusion reached that it is a deposit of fatty nature, probably akin to suberin (Priestley(10)). In view of the importance of the question the matter will be rediscussed here in the light of further evidence. The staining reactions of the deposit certainly suggest the presence of fats. Thus it frequently stains well with Sudan III (used throughout the work in a 0·2 per cent. solution in alcohol mixed with an equal volume of glycerine; the sections are warmed in this reagent upon a microscopic slide until the alcohol commences to boil), but it is usually a browner red with the reagent than the typical red stain given by the suberin lamellae of the true cork cells that are formed later below this deposit. With osmic acid the deposit blackens slowly so that the presence of unsaturated fatty acids may be suspected. The Sudan III reaction is best given by a glyceride or neutral fat; while free fatty acids seem to stain very ineffectively with this reagent when it is used for the microchemical examination of tissue. The presence of glyceride and of unsaturated fatty acids is therefore indicated by these reactions. Very interesting further evidence is supplied by the behaviour of sections stained in

Grubler's Nile blue sulphate (1 per cent. aqueous solution) followed by washing in *very dilute* alkali or in aqueous sodium carbonate (1·5 per cent.). The suberin deposit then shows two regions, an outer region which is pink or mauve in colour, and an inner region running right across the deposit where the stain remains deep blue.

As the salts of Nile blue are deep blue in colour whilst the free base is red, this reaction certainly suggests that the inner layers of the deposit contain considerably more free acid than the outer portion. Staining experiments with indicators have also suggested that the inner portion of this deposit is more acid in reaction than the tissue within, thus the deposit stains deep red with methyl red (P_H 4·4–6), whilst the cells within give a yellow reaction.

The deposit also stains very deeply with many stains that have been successfully used for suberin, notably gentian violet and safranin, but these staining reactions though useful in investigating the distribution of the deposit cannot supply much evidence as to its nature. The deposit is soluble in hot alkalis, disappearing fairly easily on boiling in 5 per cent. aqueous potash and still more easily in alcoholic potash. After its removal the cellulose walls in this region give the blue reaction with chloriodide of zinc (used according to Artschwager's method(2)) but so long as the deposit is present the walls covered by it fail to give the cellulose reactions and resist hydrolysis by concentrated sulphuric acid. These reactions are clear evidence for the presence of some fatty substances in the deposit; doubtless other pigmented substances are present with them, derived probably from oxidation proceeding in the injured or dying cells. Additional evidence for the presence of fatty substances is also supplied by the following experiments.

It was thought that if fatty acids collecting at the cut surface were responsible for the deposit, then some evidence of the presence of these fatty acids would be obtained by placing the cut surface at once in contact with aqueous alkali. On May 22nd ten potatoes were halved, one set of halves thus obtained was cut into thin slices and these were covered with an aqueous solution of potassium hydrogen carbonate in a shallow dish. The other set was similarly sliced and immersed in a solution of calcium hydrogen carbonate. After half an hour the liquid was poured off from each set and fresh solutions of the same kind added and allowed to cover the slices for 36 hours.

The potassium solutions collected were fairly clear, they were filtered, the filtrate acidified and in each case extracted with pure ether (redistilled from stick potash). On evaporation of the ether a very small quantity

of fatty acid was left, 0·008 gm. from the two filtrates together, slightly more being obtained from the first extract than from the second.

The calcium solutions contained a distinct precipitate and after filtration both precipitate and filtrate were acidified and the acid solution extracted with pure ether. In this case 0·01 gm. of fatty acid was obtained from the acidified precipitate and a negligible quantity (0·0001 gm.) from the acidified filtrate. As in the other case the greater yield of fatty acid was obtained from the liquid poured off at the end of the first half hour.

These experiments show that at the exposed surface of cut potatoes, fatty acids forming water-soluble potassium salts and insoluble calcium soaps collect within the first half hour after cutting. The quantities are very small, and apparently under the condition of the experiment new supplies do not continue to diffuse to the cut surface. These results are in agreement with the very extensive data now provided by Hansteen Cranner's investigations (4, 5, 6), which seem to place beyond doubt the presence of fatty substances in the wall and surface layers of the protoplasts of typical parenchymatous tissues. The experiments that are now to be discussed show that the conditions under which this deposit forms at the cut surface are such as would favour the oxidation and condensation of fatty acids to suberin-like products.

The Conditions of Formation of the Deposit.

A review of the earlier experiments dealing with the subject (12) showed that the evidence was strong for concluding that this deposit would be formed only in the presence of air. This conclusion was supported by our own experiments with cut surfaces left under boiled water, or under some substance preventing access of air, such as paraffin wax, when the deposit did not form.

On March 23rd potatoes were cut across under water and the cut pieces immediately placed in an atmosphere of hydrogen. They were left in this atmosphere until April 2nd, other pieces of the same potatoes being under another bell-jar in air. On April 2nd, when the pieces in the air had the normal deposit over two layers of cells and the beginnings of a cork phellogen visible beneath, the pieces in hydrogen were also examined. Microchemical reactions for fats were obtained from the contents of the cells near the cut surface but the walls of the cells appeared to be free from the usual brown deposit and with very few exceptions gave no reaction for fat. These pieces were then taken out of the hydrogen and left in moist air until April 5th when the fat deposit

could be found on the walls of one to two layers of cells, and a cork phellogen, absent on April 2nd, was also appearing in various places beneath this layer.

This deposit upon the walls of some layers of cells beneath the cut surface exposed in air has now been seen on very many occasions. It invariably precedes the appearance of the cork phellogen, it forms in air whether the cut surface is in the light or in darkness, and it forms in damp or dry atmospheres though in sunlight or a very dry atmosphere it is irregular in distribution for reasons that are discussed later. Its rate of formation varies with the variety of potato, with temperature and probably with other conditions, but all attempts to obtain a quantitative expression of its rate of formation have so far failed, as the appearance and formation of the deposit depend upon microchemical observation that cannot be made sufficiently precise. This is unfortunate as undoubtedly it is a point of considerable practical importance to know after what time in a given variety under given conditions this layer may be dense enough to prevent the entrance of pathogenic organisms. Observation suggests however, that the rate of formation of this layer in a variety is governed by the same factors that later determine the rate of formation of the cork meristem. As the latter rate admits of rough quantitative expression, the data given later for cork formation probably indicate the relative speed at which the same varieties produce the first barrier between injured tissue and external environment.

If the observations and experiments described above and previously summarised are considered as a whole they provide a considerable body of evidence for regarding this initial barrier as a deposit of fatty substances, containing some neutral fat but in the main free fatty acids; these substances accumulate on the cell walls as they oxidise and condense into larger molecular aggregates relatively insoluble in fatty solvents and removed only by boiling in aqueous or alcoholic potash. These oxidation and condensation products form a solid film which is normally continuous and unbroken at a certain depth within the injured surface. This solid film has many of the properties of a suberin lamella, properties that have been considered elsewhere (Priestley and North (11)), and will not only prove an effective barrier to the entry of most pathogenic organisms but will also restrict materially the loss of water by evaporation. Gaseous exchange such as is essential to the maintenance of aerobic respiration will also be impeded by this layer.

II. The Cork Meristem.

Within a few days of the formation of the suberin or fatty deposit described in the previous section, the cells just within the surface thus blocked show further changes. In the first place they rapidly lose their starch content, and judging by staining reactions a certain amount of starch is being converted into fatty acid. There then arises within this region by divisions running roughly parallel to the blocked surface, a layer of actively dividing cells. The outer cells of this meristem soon lose their original dense protoplasmic contents which evidently

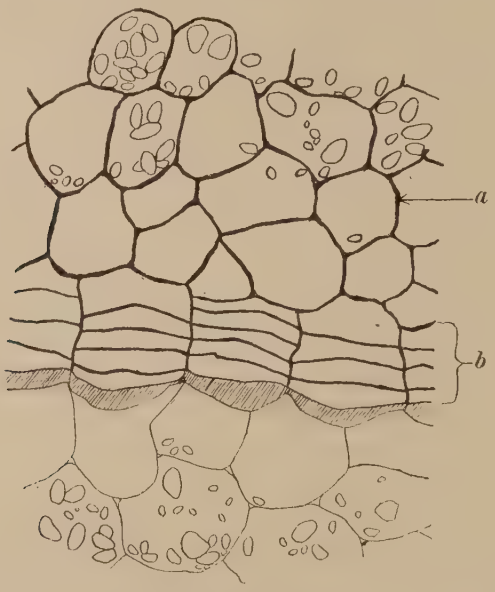

Fig. 1. Tinwald's Perfection. Section at right angles to cut surface 12 days after cutting, the cut surface uppermost.

undergo rapid chemical change. Substances partly fatty in nature are produced and these are deposited all round the wall of each individual cell as a characteristic suberin lamella, while the original thin wall, of doubtful constitution but probably containing relatively little cellulose, is also impregnated with the deposit, which gives in the most definite manner all the microchemical reactions of suberin. Thus below the original surface blocked with a fatty, suberin-like deposit (Fig. 1 *a*), there is now a new and much more stable barrier established cutting off the intercellular spaces within from the external air. This new barrier consists of a continuous sheet of tabular-shaped cells, closely joined

together without intervening air spaces (Fig. 1 *b*). Beneath the layer of cork cells so formed, the inner layer of the original meristem remains still active as the cork phellogen, so that for some time the number of layers of cork continues to increase.

The blocking of the intercellular spaces by the original fatty deposit is an essential antecedent to the formation of the cork phellogen, but the reasons for this causal relation still require considerable elucidation. Experiment shows clearly that if the blocking of the parenchymatous tissues be prevented, *e.g.* by immersing the cut surface under water or by exposure in an atmosphere of hydrogen, then the phellogen fails to appear. If on the other hand the cut surface is blocked artificially, although the natural oxidation processes causing the deposit of suberin may thus be prevented, nevertheless beneath the blocked surface a phellogen appears (Priestley and Woffenden(12)), and a considerable number of layers of unsuberised regular brick-shaped cells are produced.

The question now arises as to what consequences may follow from the original blocking of a cut parenchymatous surface. The first blocking suggests that a slight flow of sap is occurring throughout the walls and intercellular spaces in the region of the cut surface. Usually this sap bathes all walls and spaces, so that as it dries in the neighbourhood of the air, the fatty residue it leaves behind forms a continuous layer and an efficient blocking surface. If the sap continues to flow, it must now begin to accumulate behind the blocked surface. Such a flow of sap certainly seems to occur from the neighbouring vascular tissues. Appel(1) pointed out, and we confirm his observation, that the bundles in the neighbourhood of the cut stain more strongly with basic dyes, especially gentian violet, this special staining power of the vascular strand disappearing further away from the cut surface. The observation is obviously suggestive in connection with the fact that the cork meristem is active sooner in the immediate vicinity of the vascular bundles. This point is well illustrated in text-fig. 2 which shows the general position of suberin deposit (*a*) and cork meristem (*b*) below the exposed surface of a potato cut across transversely. It will be seen that both these layers approach nearest to the surface in the immediate neighbourhood of the vascular tissue (*c*).

That the cell divisions in the meristem depend upon such a sap supply is particularly emphasized by Haberlandt's experiments(7), in which the capacity of very small pieces of potato tissue to give rise to cell divisions was under examination. These experiments show clearly that some sub-

stance necessary for this purpose diffuses from the phloem of the vascular tissue, and that in a small piece of potato tissue, if phloem is absent, the concentration of this substance in the sap irrigating the isolated piece of parenchyma is inadequate to promote cell division. Haberlandt speaks of this substance as a hormone, which stimulates cells to cell division; its nature remains as yet completely unknown.

The position of the meristem in the cut potato suggests that this substance accumulates in the walls and intercellular spaces behind the blocked surface, diffusing slowly there from the vascular bundles. In the case of cork formation on growing herbaceous stems evidence has been given elsewhere (12) for attributing the transport of this substance to the flow of sap from the xylem of the vascular bundle. In the case

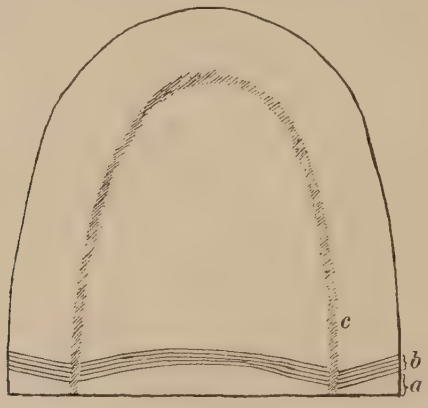

Fig. 2. Plan of cut potato—showing healing of cut surface.

of the cut potato tuber no evidence has been obtained that there is any active flow of sap along the xylem of the tuber's vascular supply. All attempts to get more active meristem formation in cut sprouted tubers, as the result of driving water under considerable pressure into the cut end of the sprout have given negative results. The evidence is quite compatible with the idea that the substance is being carried to the cut surface by slow diffusion from the phloem.

At the same time the evidence is extraordinarily convincing that the original suberin deposit and the subsequent meristem are both formed as the result of something slowly diffusing *through the water* held in the walls and intercellular spaces. Thus if potato tubers are cut across in sunlight and left exposed in dry air for a few hours, instead of a continuous deposit of suberin, small brown patches are formed here and there

at different depths below the cut surface. Appel(1), who first noticed this phenomenon, ascribed it to the lack of oxygen due to the difficulty in its diffusion through the drying surface, but the true interpretation is undoubtedly that the sap containing the fatty substances has never been able to form a continuous layer but has dried up rapidly leaving separated patches of fatty substance to be subsequently oxidised. This patchy layer of suberin is followed later by an irregular formation of meristem when the cut surface is in moister surroundings, because the new supply of sap as it oozes from the tissues and from the vascular bundles in particular, cannot form a continuous layer beneath a discontinuous blocked surface.

The supply of sap from the healthy tissues and notably from the

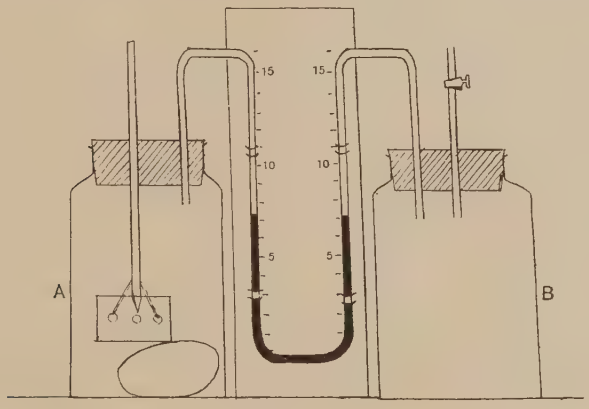

Fig. 3.

vascular bundles of those tissues, is one factor in the production of the cork meristem but it is not the only one. Another factor of importance is undoubtedly the production of fatty acids below the blocked surface as the result of decomposition of the carbohydrate reserves under anaerobic conditions. The disappearance of starch is always associated with the appearance of free fatty acid beneath the blocked surface. The following experiment is somewhat instructive in this connection. Potatoes are placed in a bottle (*A* of text-fig. 3) connected by an oil manometer with another bottle *B*, the volume of air in *B* being adjustable by opening or closing a tap. The whole apparatus is placed in an incubator and adjusted until the manometer reading remains steady. By means of a Gillette razor blade inserted air tight through the rubber cork in *A* the potatoes are now cut repeatedly and the incubator closed and pressure

changes observed through the glass door, whilst the apparatus is maintained at constant temperature.

The changes in pressure that follow are always of the same type. First for a few hours the pressure in *A* falls, the oil rising in the left-hand arm of the manometer. This may possibly be associated with the intake of oxygen during the oxidation of the unsaturated linkages in the fatty substances in the exposed tissue. But after the first few hours the level of the oil falls in the left-hand tube and continues to do so for the next 24 hours; during this period more carbon dioxide is presumably being given out than oxygen absorbed. In the region below the cut surface during this period the rapid entry of oxygen is prevented as the fatty layer deposits, and below this layer the anaerobic decomposition of sugar with formation of fatty acid probably takes place.

Chemical considerations (Leathes(9) *loc. cit.* p. 107) suggest that the conversion of glucose into fatty acids can be accomplished without the addition of further oxygen, carbon dioxide being given off in the process and energy released, although the remaining carbon is now obtained as fatty acid in which the energy stored in calories per gram is relatively greater than in the original carbohydrate reserve.

The presence of these fatty acids below the blocked surface undoubtedly causes the local production of a medium bathing the adjoining protoplasts which is relatively more acid than the sap of the parenchyma itself. Observations upon different species of plants made by Mr Herklots in this department with the use of indicators show that this is a common phenomenon during cork formation and that when such a gradient of reaction (P_H) arises, a meristem frequently separates the tissues of different reaction. The same phenomenon is shown by the normal cambium of the vascular bundle arising between the phloem, a relatively alkaline tissue as Sachs observed long ago(13), and the relatively acid xylem (Atkins(3)). This raises a very general problem which it is hoped to deal with more adequately in a later communication from this laboratory.

Haberlandt(8) has invoked another factor in this connection which might replace the acid reacting sap below the blocked surface. He suggests that another hormone, or cell division stimulating substance is released from the wounded cells at the cut surface. But the cork meristem frequently appears for the first time at the end of seven or eight days, beneath a surface that has been blocked off from the injured cells at the surface for four or five days already, and in the subsequent weeks when cell division is active no such supply of "wound hormone" could

reach the dividing phellogen. Furthermore, experiments in which the tubers have been cut across below a strong jet of water and half the cut surface held in a clamp under the jet, in one case for twenty minutes and in another for $2\frac{1}{2}$ hours, have failed to demonstrate any retardation of cell division as a result of leaching away of the wound hormone. Thus on March 23rd potatoes were cut open in this manner and left under the jet of water for $2\frac{1}{2}$ hours, the control half of the tuber being left in moist air in each case. On April 3rd both treated and control surfaces showed six layers of meristem (two suberised and four unsuberised in each case). Haberlandt also failed to obtain evidence that these wound hormones could be washed out from the cut cells of the potato. His positive results as the result of similar experiments with Kohlrabi seem better explained by the assumption that in this plant the washing of the surface with water interfered with its subsequent blocking, owing to the leaching away of the necessary fatty substances. (See Priestley and Woffenden (12).)

The activity of the phellogen produces a number of rows of very regular cells. The earliest formed rows to the outside are soon suberised and suberisation progresses slowly inwards, until as the rate of formation of the cells slows down the process of suberisation overtakes it and all cells except perhaps the innermost are seen to be suberised. It is a relatively simple matter to count the number of rows of cells thus present in the cork layer. Preliminary experiment showed that with uniform seed tubers of a given potato variety cut and placed under uniform conditions, cork formation proceeded at practically the same stage, as estimated by the number of layers of suberised and unsuberised cells formed. Some 24 tubers of the same variety were thus examined, sections being taken from the same region, the centre of the tuber in each case, so as to avoid the irregularity introduced by the neighbourhood of vascular tissue. The results obtained having varied very little, a comparison was now extended over a fairly representative series of varieties, the material being all Scotch seed obtained from Messrs Dobbie and Co., Edinburgh. The results are given in the accompanying table (Table I). The tubers in this experiment were left in the laboratory for 17 days under bell jars on moist filter paper. After the first few days, precautions were taken to protect them from direct sunlight, but some irregularities in these early results may be due to the irregular drying effect of occasional periods of exposure to the sun in the first two or three days.

Direct sunlight was avoided in the second series of experiments,

Table I.

Potatoes cut and left in laboratory 17 days.

Variety	Remarks	Numbers of layers of cells: Suberised meristem	Un-suberised meristem	Total meristem	Average of total meristem	Layers of previously suberised cells
Dargill Early	Variable meristem, not very regular. Begins 2nd row from surface	0–3	0–3	0–6	3	2
Rhoderick Dhu	Fairly regular. Varies in depth between 2nd and 4th row from surface	3–4	1	4–5	4	2–4
K. of K.	Fairly regular. Begins well below surface 2–3 cells deep	2–4	1–3	4–6	5	3
Crusader	Fairly regular. Varies in depth from surface	2–4	2–5	4–7	5	2
Arran Comrade	Irregular, occasionally no meristem. Occurs at varying depths 1st–3rd row. Meristem good when in 1st row	0–4	0–3	0–7	5	2–3
King Edward VII	Regular. In 2nd row of cells	2–4	2–4 (2–9 near veins)	3–11	5	2
America	Very variable. Practically no meristem in some but deep suberisation. Others regular meristem in 2nd or 3rd row	0–4	0–5	0–7	0 or 5	3
Kerr's Pink (W)	Variable. In one, regular meristem in 1st cell deep. Others irregular, at different depths, and 2–3 cells below surface	0–3	0–3	0–6	4	1–3
Kerr's Pink	Regular meristem, in 1st or 2nd row	2–4	1–4	3–6	5	1 (rarely 2)
Arran Chief (W)	Regular. Very near surface in 1st row	2–3	1–3	3–6	4	1
Arran Chief	Fairly regular in 1st or 2nd row	1–3	1–4	1–6	4	1–2
Bishop (W)	Fairly regular 2nd row	2–4	1–2	3–5	5	1–2
Bishop	Irregular. Occasionally no meristem, occurs generally 3rd row	0–3	0	0–2	3	3
Tinwald's Perfection	Fairly regular. Generally 2nd row deep	2–4	1–3	3–6	5	2
Immune Ashleaf	Fairly regular. Generally 2nd or 3rd row	0–2	0–3	0–5	4	2–3
Champion (W)	Regular, usually 2nd row deep	2–4	2–6	3–8	5	1–2
Great Scot	Irregular meristem. Occasionally none. Sometimes near surface, sometimes 3rd row	0–3	0–7	0–9	4	1–3
Resistant Snowdrop	Fairly regular. At different depths 1st or 2nd, occasionally 3rd row	2–4	1–4	3–7	5	1–2 (rarely 3)
Arran Rose	Regular, but of varying thickness, 2nd row	2–6	1–3	3–8	5–6	2
Majestic	Irregular or regular. Always near surface	2–3 (very rarely 0)	1 (–4 near veins)	3–6 (0)	3	1–2

(W) refers to seed tubers, grown from Scotch seed during 1921 at the experimental ground at Weetwood, Leeds.

Table II.

March 15-27th in greenhouse.

Variety	Remarks	Suberised meristem	Unsuberised	Average amount meristem	Depth of previous suberisation
Dargill Early	Fairly regular meristem	3–4	2–3	5	3–4
Roderick Dhu	Regular meristem	3–5	1–3	5	3
Crusader	Very little meristem—generally 0	0–1	0–3	2 (0)	4
K. of K.	Very regular meristem	3–5	0–1	5	2–3
Arran Comrade	Fairly regular	2–3	2	4–5	4
King Edward VII	Very poor and irregular	0–2	0–1	0–3	2
America	Meristem, where present is regular and usually quite near surface. In some sections have deeper suberisation (3) and little or no meristem	3 (occas. 0)	3 (occas. 0)	5	1–3
Kerr's Pink (W)	Regular (with few exceptions)	2–4	1–3	5	2–3
,, ,,	Not much difference from above	3	2	5	1–3
Arran Chief (W)	Generally no meristem	0–3	0–1	0 or 2	2–3
,, ,,	Fairly regular	1–4	1–2	1 or 4	3–4
Bishop (W)	Very variable, most have no meristem	0–4	0–1	4–5 or 0	2–3
,,	Practically no meristem	0–2	0–1	0–2	3
Tinwald's Perfection	Regular	2–5	2–3	5	2
Immune Ashleaf	Not very regular. Occasionally absent	0–4	0–4	0 or 3	2–3
Champion (W)	Regular	3–4	0–1	4	3
Great Scot	Irregular, occasionally 0	0–4	0–1	3–4	3
Resistant Snowdrop	Regular	3–5	1–2	5	3
Arran Rose	Fairly regular	2–5	2–3	5	2–3
Majestic	Irregular	0–3	0–2	0 or 2	2

Table III.

Fifteen days, March 22nd–April 6th in greenhouse.

	Sprouted			Unsprouted		
	Suberised	Unsuberised	Average total	Suberised	Unsuberised	Average total
Dargill Early	0–4	0–1	0–2	0–3	0–1	0–2
Roderick Dhu	0–3	0	0–2	0–1	0	0
Crusader	2–4	0–1	3–4	2–4	0–1	3
K. of K.	0–2	0–1	0 or 2	0–2	0–1	0–1
Arran Comrade	0–5	0–2	2–3	0–5	0–1	2–3
King Edward VII	2–4	0–3	4–5	2–4	0–2	4
America	3–6	2	6	2–4	2–3	5–6
Kerr's Pink	2–4	0–2	4	2–4	0–2	4
Arran Chief	2–5	0–1	4	1–5	0–2	4
Bishop	0–3	0	0	0–3	0	0
Tinwald's Perfection	2–5	1–3	5	2–4	0–2	4
Immune Ashleaf	1–4	0–2	3–4	1–4	0–2	3–4
Great Scot	2–5	0–2	5–6	2–5	0–2	5
Resistant Snowdrop	1–4	0–1	3–4	1–3	0–2	3–4
Arran Rose	2–6	1–2	4–5	1–4	0–1	3–4
Majestic	0–2	0–1	0 or 2	0–2	0–1	0 or 2

Table IV.

Twelve days at 25° C. in incubator.

	Suberised	Unsuberised	Average total	Previously suberised
Dargill Early	2–4	1–3	5	2
Rhoderick Dhu	3–6	0–1	6	1–2
Crusader	0–3	0–1	2	3
K. of K.	0–1	0	0	2
Arran Comrade	0–4	0–2	0 or 3	2–3
King Edward VII	2–4	0–2	3–4	1–2
America	2–4	1–3	5–6	2
Kerr's Pink	1–6	0–3	4	2
Arran Chief	0–2	0–2	0 or 3	1
Bishop	1–2	1–3	2–3	2
Tinwald's Perfection	2–6	0–2	5–6	3
Immune Ashleaf	0–3	0–2	0 or 3	1–3
Great Scot	3–5	0–1	5–6	1–3
Resistant Snowdrop	2–6	0–1	4–5	2
Arran Rose	1–4	0–2	4	2
Majestic	1–3	1–2	3	1–2

Table V.

		Twelve days at 25° C.			Twelve days at 15° C.		
Variety	Remarks	Suberised meristem	Unsuberised meristem	Average amount meristem	Suberised meristem	Unsuberised meristem	Average amount meristem
Dargill Early	Fairly regular	2–5	0–2	4	0–1 occas.	1–3	2
Rhoderick Dhu	Regular	0–3	1–2	3	0	0–3	0–2
Crusader	— —	1–3	2	4	0–1 rare	0–3	0 or 2–3
K. of K.	At 25° C. 2–4 rows of cells at surface were unsuberised and below these were 2 rows suberised cells. At 15° 1–2 unsuberised, 2 suberised	0–1 occas.	0	0	0	0	0
Arran Comrade	Very variable amounts of meristem	0–3	0–2	0 or 3–4	0	0–3	0 or 2
King Edward VII	Fairly regular but occasionally none	0–3	0–2	3 occas. 0	0	0–2	0 or 2
America	Very good and regular	2–3	2–3	5–6	0	2–3	2
Kerr's Pink	Compressed appearance of meristem	0–3	0–2	4 occas. 0	0	0–2	0 or 2
Arran Chief	Near surface, irregular	0–3	0–2	0 or 3 rare	0	0–2	1–2
Bishop	— —	0–2	0–2	0–3	0	0 or 2	0
Tinwald's Perfection	Regular	2–6	1–3	5–6	0–1	0–3	2
Immune Ashleaf	Very variable. Regular in places. None or irregular in others	0–4	0–2	0 or 3	0	0 occas. 2	0
Great Scot	Fairly regular	2–5	0–3	5	0	2–4	3
Majestic	— —	0–2	0–1	0 or 2	0–1 rare	0–2	0 or 2

summarised in Table II, which were carried out in a similar manner, the cut tubers being left from March 15th until March 27th under bell jars in the greenhouse. The most striking feature emerging from these two tables, and seen also in the later tables, seems to be the poor production of cork meristem shown by the varieties Majestic and King Edward VII. Bishop, though irregular, is also very unsatisfactory, though it is interesting to note that tubers grown from Scotch seed for one year at Weetwood, Leeds, are producing better cork than seed tubers fresh from Scotland. So far as we can gather these three varieties, especially Majestic, would all be picked out by practical growers as relatively unsuitable for propagation by sets because they are "bleeders," *i.e.* found by experience not to heal well after cutting.

Potatoes are frequently sprouted before cutting, but to judge from the data supplied in Table III the previous sprouting has little effect upon the rate of healing of the tuber. These tubers were left from March 22nd to April 6th in the greenhouse and comparison is made between the half of the tuber with the sprout and the half without. The experiments so far described have been carried out at room temperature, without special precautions to keep it constant or to record it. Their results are in accordance with the experimental data supplied in Table IV, for cut potatoes kept for 12 days in an incubator at a constant temperature of 25° C. Finally, Table V shows the effect of temperature on the rate of formation of the meristem. It is interesting to see that the rate of formation appears to be approximately double when the temperature rises from 15° C. to 25° C. This temperature effect corresponds with the change of rate of a chemical reaction with temperature, and suggests that a chemical reaction may be the governing factor determining the rate at which the complex process of cork formation is proceeding.

Throughout these comparisons of cork formation in different varieties every precaution was taken to have conditions as constant as possible and tubers as comparable. In the case of three varieties (Kerr's Pink, Arran Comrade, and Tinwald's Perfection) experiments were carried out to see whether in relatively large and small potatoes appreciable differences in rate of cork formation were found. Details are not given but the result showed that no appreciable difference is to be found between large and small tubers when cut just before planting in the spring. On the contrary, some observations by Mr Cheveley in this department show that if the tubers be tested immediately after lifting in the summer then the small tubers are more active in cork formation.

In all these experiments the tubers have been cut across transversely. Some experiments were carried out to compare the behaviour of larger

or smaller pieces of tuber, but the practical result appears to be that pieces such as are likely to be met with in practice, if cut from the same tuber have practically the same power of healing. When very small pieces are cut their behaviour is very different and depends upon the presence or absence of the phloem of the vascular bundle. The question has been fully explored experimentally by Haberlandt (7).

III. The Practice of cutting Potato Sets.

The data given in the previous sections may obviously throw some light on the best conditions under which potatoes are propagated from cut sets. The advisability of this practice is not under discussion. For various reasons it is still frequently employed and it is important to be able to define the most suitable practical conditions under which to carry it out.

When the cut tuber is placed in the ground, the most essential point is that it should be resistant to the entry of pathogenic organisms. A freshly cut surface is undoubtedly not resistant to organisms that penetrate the tissue rapidly; after the first layer of suberin has been deposited the surface is probably well protected provided the layer is continuous. To judge from the experiments described, so long as air has access to the cut surface this deposit of suberin will form, and provided the atmosphere is not too dry it will be formed in a continuous sheet. It appears then that tubers should be cut, left for a day or two in any moist warm place, preferably not piled in heaps but loosely spread out, and then planted: but unless the soil is very heavily infected with some pathogenic organisms the cut tubers would probably heal when placed directly in the soil, with very few casualties owing to disease reaching the healthy tissue before it is barred out by suberisation.

The Undesirability of Cutting in Sunlight.

Experiments have indicated clearly that if the potato tuber is cut and left for a time in sunlight, the continuous deposit of suberin is lacking and the potato therefore remains much more susceptible to disease. In cases such as growers report, where fields planted with potato sets of a variety like Majestic, have proved a complete failure, it would probably be well worth while to see whether this has been the cause of failure. During our own numerous experiments, notes have been kept of the occasions where cut tubers have been lost owing to fungus attack. In every case without exception, cutting in sunlight or exposure to too dry an atmosphere can be traced as the most probable cause. The dry

atmosphere is particularly dangerous when the general temperature is low, and the suberin layer in consequence relatively slow in forming.

Some practical trials on this point are in progress, and the following results of a small scale trial at Doncaster by Mr G. C. Johnson, Instructor in Practical Horticulture, University of Leeds, are at least suggestive.

Variety: Great Scot. (1) Sets cut and exposed to bright sun, planted after 24 hours.
Yield 9 tons per acre.
(2) Sets cut and left in warm, damp, shady place for 24 hours.
Yield 17 tons per acre.
(3) Sets cut and planted immediately.
Yield 13 tons per acre.
(4) Control: whole sets seed size, planted.
Yield 18 tons per acre.

Apart from the need for the avoidance of direct sunlight or too dry an atmosphere for the cut sets prior to planting, and the advantage of an interval after cutting, before the sets are planted, wide difference in practical treatment would seem permissible.

The Practice of Liming Cut Sets.

It is a frequent practice to place the sets in powdered lime immediately after cutting, and considerable attention has been given to this question to see if the somewhat troublesome operation is justified by its results. In the first place we have been unable to ascertain definitely whether slaked lime or caustic lime should be used in this operation, potato growers having been found who are equally emphatic in favour of each of these alternatives respectively. We have therefore carried out experiments with both slaked and caustic lime, more frequently with caustic lime as the balance of evidence seemed to be in favour of this treatment. It might be anticipated that caustic lime would exert a considerable drying effect, which, if it did not prevent a continuous deposit of the fat might be of some value as favouring the change from the fat to the suberin, a change which undoubtedly is associated with loss of water from the fat (Priestley (10)). In fact, however, we have not been able to find that the caustic lime has any more effect upon the deposit of the suberin than the slaked lime, and neither form of lime seems to aid the formation of the normal suberin deposit. On the contrary the suberin layer is micro-chemically much more difficult to detect in the limed potatoes probably because, as might be anticipated from the experiment on p. 99, the fatty acid instead of condensing to suberin is to a considerable extent now precipitated at the surface of the injury as a calcium soap.

Observations made by growers had suggested to us that the limed sets lost less water in the air than the unlimed. These observations seemed to indicate that a better deposit of suberin had formed as a result of liming, but as the result of experiment we are driven to the opposite conclusion, viz. that limed sets lose more water than unlimed and that this is due to the deposition of the fatty acid in part as a calcium soap and the relatively weak blocking layer of suberin then developed. Experiment upon the loss of water from limed and unlimed sets prove difficult to carry out in a way free from objections. Experience showed that comparison should be made between pieces from the same tuber, left to dry under strictly comparable conditions, apart from the presence or absence of lime. The pieces needed to be of the same thickness and the loss of weight resulting from evaporation had to be calculated upon the area of cut surface exposed. There would be a certain loss of moisture from the normal uncut surface and in many experiments these surfaces were sealed by dipping in a paraffin wax of low melting point, in others cubes of potato tissue were used on which none of the original surface layer remained.

The final conclusion of a large amount of experiment upon this subject is that, under the conditions existing in practice, the procedure of liming, either with slaked or unslaked lime, has no practical value and that provided tubers are not cut in sunlight or left in too dry an atmosphere, a day or two's delay before planting is a more effective method of protection against disease. Similarly Shapovalov and Edson(14) conclude that the American practice of dusting with sulphur is unnecessary and drying of the tissues the thing to be avoided.

These practical conclusions are at present the subject of further experimental trial at the Manor Farm, Garforth, and at Doncaster, through the cooperation of the staff of the Agricultural Department of the University of Leeds. In view of the simplicity of the practical procedure that is suggested as most advisable for cutting sets, it is hoped that other agricultural experiment centres will compare the value of cut sets left under moist or dry conditions before planting. If our conclusions are supported by such experiment a hitherto unsuspected source of serious loss in present day practice with cut sets may be eliminated in future.

Summary.

(1) The sequence of events during the healing of the cut surface of a potato tuber is carefully traced.

Apart from unessential colour changes, the first process in the healing of the tuber is the deposit of a fatty "suberin" layer, formed by the

oxidation and condensation of fatty substances depositing from the sap drying up in the tissues at the cut surface.

(2) The condition essential to the formation of this layer is access of oxygen.

(3) If the cut surface is exposed in a moist atmosphere the layer is continuous; in a dry atmosphere, especially in sunlight, the layer may not be continuous.

(4) This layer forms within 24 to 48 hours. A few days later cork is formed below this suberin deposit as the result of cell divisions in an actively dividing layer, the cork phellogen.

(5) The essential factors promoting the activity of this phellogen appear to be the accumulation behind the blocked surface of sap containing substances diffusing from the vascular bundles, and the production of an acid reaction just below the blocked surface by the anaerobic conversion of sugars into fatty acids.

(6) The activity of the cork phellogen may be roughly estimated by the number of layers of cork produced. Comparative data are presented for a number of varieties of potato which show that Majestic, King Edward VII and Bishop are particularly deficient in this important activity connected with the healing of wounds.

(7) The bearing of these facts as to the process of wound healing is considered in relation to the practice of propagation by potato sets and two practical conclusions drawn. (1) Potato tubers should not be cut in sunlight or left in too dry a place. (2) The practice of liming potato sets appears to be without justification so far as its use in promoting the healing of the wound is concerned.

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EXPERIMENTS ON THE FEEDING OF EMBRYONATED EGGS OF *ASCARIS MEGALOCEPHALA* TO DOMESTICATED ANIMALS

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Introduction.

Researches carried out during the last few years on the life-history of certain species of Ascaris by a number of workers, Stewart(10), Ransom and Foster(8), Ransom and Cram(7), Yoshida(11), Fülleborn(1, 2, 3), Schwartz(9), Ortlepp(6), have shown that when embryonated eggs are fed to the normal host or to certain other laboratory animals, abnormal hosts, the contained embryos are set free when the eggs reach the intestine, and the larvae then migrate through the intestinal wall into the lymph vessels or the blood stream. In the latter they get carried to the liver and thence through the heart to the lungs.

By their presence in the lungs they may give rise to definite and well-marked pneumonic symptoms to which the animal may succumb. This holds good for both the normal and the abnormal host. For example, it has been found that the larvae of *A. lumbricoides*, normal to the pig and man, can set up pneumonia not only in pigs but in rats, mice, guinea-pigs, rabbits and goats; whilst Ortlepp(6) has found that the larvae of two species of Ascarids, *Polydephis attenuata* and *Ophidascaris filaria* from the Python, a cold-blooded vertebrate, can cause fatal pneumonia in a warm-blooded vertebrate such as a mouse.

Besides the extreme theoretical interest of these results, they are of great practical importance in that they reveal the possibility of the infestation of domesticated animals by the larvae of parasites not normally occurring in them.

The dog, the pig, the ox and the horse are specific hosts for Ascaris, the larvae of which, in the case of the first three of the above named, produce grave pulmonary lesions in experimental animals.

The point of economic importance was to ascertain how far the larvae of these parasites could produce pneumonic symptoms in sheep, and this

led us to take up, in the first instance, the study of the Ascaris of the horse, *A. megalocephala*, as no work on these lines had been prosecuted on this species. The horse is herbivorous and it seemed within the realms of possibility that other herbivorous animals such as lambs might run the risk of pulmonary trouble simply by feeding on pastures infected with the embryonated eggs of *A. megalocephala*.

The results recorded hereafter show that the larvae of this species of Ascaris can produce pulmonary lesions in laboratory animals and that consequently *A. megalocephala* comes into line with the other Ascaris species in which migration through the lungs is a normal occurrence.

The experiments on two lambs described in the present paper were carried out at the farm of the Rothamsted Experimental Station, Harpenden. On behalf of Professor R. T. Leiper and myself I desire to express our indebtedness to Sir John Russell, F.R.S., Director of the Station, and to Mr S. J. K. Eames, the Farm Manager, for kindly placing the necessary accommodation and working facilities at our disposal.

Material.

Specimens of female *A. megalocephala* were obtained from various sources, and after opening up the body cavity the two uteri were removed. As a rule the anterior third of each was taken and the remainder rejected as probably containing eggs incapable of completing their development. The procedure recommended by Ransom and Foster(8) for the culture of the eggs was followed. The portions of uterus were put into 2 per cent. formalin and cut into small pieces, and then lightly rubbed up in a mortar so as to force out the eggs and break up the larger egg masses. The resulting mixture was put into Petri dishes and incubated sometimes at 22° C. and sometimes at about 30° C. In nearly all cases there was a varying proportion of eggs, which failed to develop, but in most cases very large numbers of embryonated eggs were obtained.

Infection Experiments.

Experiment 1. Two mice were fed with biscuit moistened with a suspension of many thousands of washed embryonated eggs. The animals had remained without food over night before being given their first feed of infective material. They were fed on two successive days and must have swallowed a very large number of embryonated eggs.

On the fourth day both of them showed signs of illness and were huddled up in the corner of their box.

On the fifth day both were found dead, with blood exuding from the

nose and mouth, which had evidently been coughed up from the lungs. On opening up the body cavity the liver and lungs were both seen to be abnormal in appearance, the lungs showing many bright red spots, whilst the liver had numerous creamy spots scattered over its surface. Press preparations of portions of both liver and lungs from the region of the diseased spots showed numerous Ascaris larvae actively moving in the tissue. One mouse was fixed entire after being skinned, and was put aside for later examination. From the other, portions of the liver and lungs were taken and fixed. These were embedded in paraffin in the usual way and sections were made which showed the larvae in the tissues.

There could be no doubt that the mice had succumbed to pneumonia set up by the larvae of *A. megalocephala* in lungs, and this positive result showed conclusively that the passage of larvae through the lungs is normal for *A. megalocephala* as for *A. lumbricoides*, *Belascaris* and *Toxascaris* spp., *Polydephis attenuata*, *Ophidascaris filaria* and *A. vitulorum*.

Experiment on skin penetration.

The following note on an experiment to test the possibility of skin penetration by the larvae of *A. megalocephala* may quite well be given here. The experiment was prompted by the fact that Yoshida (11) claims to have infected a guinea-pig through the skin with larvae of *A. lumbricoides* obtained from the liver of another experimental guinea-pig, and placed on the shaved skin of the abdomen and in the region of the shoulder. Another Japanese worker, Asada, is cited by Fülleborn (2) as having succeeded in getting larvae of *A. lumbricoides* to penetrate the intact skin of mice, rats and guinea-pigs. Ortlepp (6) found that the larvae of the two species of Ascarid *Polydephis attenuata* and *Ophidascaris filaria* were incapable of penetrating intact skin when tested by the method revised by the present writer, Goodey (4, 5). Ortlepp, by the way, gives the wrong reference in his text to Fülleborn's paper; it should be Fülleborn (1921) not (1922).

In the experiment recorded here the larvae of *A. megalocephala* were obtained from the droppings of a mouse three and a half hours after it had been fed with a heavy dose of embryonated eggs. The droppings were teased up in a little distilled water and the larvae which swam about freely were picked up by means of a capillary pipette. In this way a fairly large number were collected in a small drop of water, which was transferred to the surface of a portion of skin removed from the abdomen of a very young rat, which had been stretched and pinned over

a hole in a piece of cork, and then floated on the surface of N. saline kept at 37° C. in the manner described by the author(4, 5).

The *A. megalocephala* larvae were seen to show a certain amount of spasmodic movement, but there was no definitely downward movement such as was observed in the experiments with larvae of *Necator americanus* described in the paper referred to above. The drop of water containing the larvae was allowed to dry up gradually and all the conditions under which larvae of *Necator americanus* penetrated the skin were arranged, but at the end of the experiment none of the larvae had penetrated, and the majority of them were removed from the skin by flooding it with a drop of water and then sucking this up in a pipette. It would thus seem that the hatched larvae of *A. magalocephala* are incapable of penetrating intact skin.

EXPERIMENTS WITH LAMBS.

Experiment 1. A male lamb, four days old, was obtained and taken to Rothamsted Experimental Station Farm, where throughout the course of the experiment it was bottle fed.

On two successive days it was fed with a heavy suspension of embryonated eggs added to the milk in the feeding bottle; many thousands of eggs were given. The weather was very cold during the period of the experiment and the lamb, although well cared for and protected from draughts, seems to have contracted a chill, for it died seven days after the first feeding with eggs.

On opening up the animal at the post mortem, the only sign of disease was seen in the left lung which was very much congested and dark red in appearance. The right lung was quite healthy and so also was the liver.

Numerous press preparations were made of portions of both lungs and liver, and were examined microscopically, but no Ascaris larvae were found.

A careful search was also made through portions of the intestine but with negative results.

Experiment 2. About ten days after the death of the first lamb another one was obtained from the same source. This was a female ten days old, and as before, it was bottle fed during the course of the experiment.

A very thick culture of washed embryonated eggs was fed to this lamb by means of a teaspoon, the masses of eggs being spooned up from a saucer. The feeding was completed by washing down with a little distilled water from a bottle. This method of giving eggs was very suc-

cessful. Fourteen days later the lamb was given a second lot of eggs by the same method, and two days after this a third feed was administered. In these three feeds the lamb received enormous numbers of embryonated eggs, the contents, in fact, of three culture dishes, each six inches in diameter, and each culture so rich in eggs that one could not see through it. It must have taken over a million eggs. The animal did not, however, at any time during the course of the experiment suffer the slightest inconvenience, and at no time did it exhibit any signs of pulmonary disturbance. Droppings were collected 24 hours after the first feed, and these were found to be extremely rich in eggs. A few free larvae were also found in the droppings, thus showing that a certain amount of hatching had taken place. Empty egg-shells and partially hatched eggs were also found.

A count was made to determine the numbers of (1) unhatched embryonated eggs, (2) partially hatched egg, and (3) empty shells. The result of the count showed that for every 100 unhatched embryonated eggs in the droppings there were about 30 partially hatched, and only five empty egg-shells.

A similar count was made on the eggs passed in the droppings collected 24 hours after the third feed. On this occasion also eggs were extremely plentiful in the faeces, and a few free larvae were also found. The proportions of unhatched embryonated, partially hatched and empty egg-shells were the same as in the previous count, *i.e.* unhatched 100, partially hatched 30, and empty shells 5.

The two counts show that comparatively few of the embryonated eggs administered had hatched out in the intestine. In view, however, of the enormous number of eggs administered in the three feedings it would not have been surprising had the lamb shown signs of pulmonary disturbance, even though only 5 per cent. of the embryonated eggs had hatched.

The results of these two experiments are interesting and lead one to the conclusion that lambs are not susceptible to pulmonary trouble due to the migrating larvae of *A. megalocephala*.

It is not claimed, however, that this is a wholly conclusive result inasmuch as two attempts to set up pulmonary symptoms in mice proved failures for some unknown reason, and in this connection it may be mentioned that some of the workers already cited have recorded negative results in some of their experiments. It is thus possible that the failure in the present instance may come in this category of experiments, though I do not think this a probable explanation.

It is interesting to note in this connection that Ransom and Foster (8), p. 42, in their one recorded experiment on a lamb which they fed with embryonated eggs of *A. suum*, do not mention the occurrence of any pneumonic symptoms, and one naturally presumes that no such symptoms manifested themselves, or they would have been noted as in the other cases where such symptoms arose.

CONCLUSIONS.

1. The larvae of *A. megalocephala* are capable of causing fatal pneumonia to mice in their migratory course through the lungs.

2. Attempts to cause pulmonary disturbance in lambs by feeding them with embryonated eggs of *A. megalocephala* proved unsuccessful and it would seem that lambs are not susceptible to such trouble. The need for further work on these lines is indicated.

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SOME PRELIMINARY INVESTIGATIONS ON THE RELATIONSHIP OF PROTOZOA TO SOIL FERTILITY WITH SPECIAL REFERENCE TO NITROGEN FIXATION

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(With 1 Diagram.)

I.

Although the presence of protozoa in soil was observed by Müller(10) as far back as 1887, real advance in our knowledge concerning their activities and importance in relation to soil fertility dates back only to 1909, when Russell and Hutchinson(13) announced their "Protozoa" theory of the partial-sterilisation of soils. This theory has been a subject of much speculation and discussion as to the nature of limiting factor.

"Protozoa" theory.

Russell and Hutchinson(13) considered "that the soil population is complex and that some of its numbers act detrimentally on the bacteria which produce plant nutrients: these detrimental forms are more readily killed than the useful bacteria, with the result that the new population produces more ammonia and nitrate than the old one. The detrimental organisms are provisionally identified with protozoa."

The evidence put forth by them in support of their theory tended to show that protozoa do work as phagocytes on soil bacteria and the increased fertility of the partially-sterilised soil was due in part to the availability of a large amount of organic matter left behind in the form of dead bodies of organisms killed by partial sterilisation.

Effect of protozoa on mass number of bacteria in soil.

Russell and Hutchinson's experiments showed a depressing effect on the mass number of bacteria when normal soil containing protozoa was added to the partially-sterilised soil. Cunningham(1) and Goodey(4) also recorded observations of similar nature and confirmed Russell and Hutchinson's views. Waksman(15) too admitted that certain of the trophic fauna acts detrimentally on soil bacteria.

Martin and Lewin (9) established that protozoon occurs normally in a trophic state in soils.

Cutler's (2) ingenious method of determining active numbers of protozoa in soil affords direct evidence that they occur in an active and trophic form, and as such are able to feed upon bacteria, bringing about a depression of their numbers.

Cutler, Crump (3) and Sandon (3*a*), in their daily counts for 365 days, have succeeded in demonstrating this depressing effect of amoebae on bacterial numbers[1].

Greig-Smith (5), on the other hand, denied the functioning of protozoa as a limiting factor to soil bacteria in the soils he examined. He suggested instead that "Agricere" (6) forms a water-proofing layer around the soil particles, and is destroyed or segregated at the points of soil particles by partial sterilisation. These conditions, however, could not be reproduced experimentally, and the wax instead of forming a water-proofing layer served as an excellent food material for the bacteria.

Sherman (14) also joined hands with Greig-Smith in opposing the phagocytic action of protozoa on soil bacteria. His results show enormous decrease in bacterial numbers in presence of protozoa in a partially-sterilised soil, yet he concludes that protozoa have no action whatsoever on soil bacteria. The figures he obtained in the case of pure cultures were very contradictory. In one test the bacterial numbers increased when protozoa were introduced, while in another similar test they decreased. The incubation period was only eight days, hardly enough to give time to protozoa to establish themselves. Evidence is also lacking to show if the so-called "active" cultures were really alive and active after the inoculation from the artificial media to soil. It will be shown later that inoculation with protozoa is not a simple matter. The types of protozoa made use of were only ciliates and flagellates, missing the most important group the "amoebae," which are more destructive and are normally present in soils.

The relationship of protozoa to the most important bacteriological processes in soil.

1. *Ammonification.*

In a partially-sterilised soil in which protozoa were absent Russell and Hutchinson (13) observed a great increase in ammonia production in comparison with the untreated soil. Cunningham's work also

[1] The inoculation experiments described by Cutler in this number of the Annals (*Ann. App. Biol.* 137) afford an interesting confirmation of this statement.

supported Russell and Hutchinson's observations. Hill(7), on the other hand, carried out a set of triplicate experiments and concluded that protozoa have no effect on ammonification. His results show 100 per cent. increase in ammonia production after 12 weeks in absence of protozoa, but this was more than offset by a difference in nitrate production. Again, however, it is not clear that the inoculation was properly carried out.

Lipman, Blair, Owen and McLean's(8) work on the possible influence of protozoa on ammonification contains large number of figures obtained by using dried blood, tankage, soluble-blood flour, cottonseed meal, soy-bean meal, wheat flour, corn meal etc., but does not clearly show whether the soil used for the experiment actually contained protozoa. From the text one finds no evidence of the existence of protozoa, either in the original soil, or the inocula used in the tests. It may be doubted also whether much reliance can be placed on results obtained with substances liable to great variations in their composition and not normally present in the soil.

2. *Nitrogen-fixation.*

The process of free nitrogen-fixation in soils is an important one, more especially in countries where on *Barani* (dependent on rain) soils of light sandy nature crops are taken year after year without any application of manure. After each crop the land is allowed to remain fallow for a considerable period—usually till the next sowing season—during which time the usual agricultural operations are carried out.

The effect of soil protozoa on this class of organisms has not been studied in detail. Hill(7) carried out an experiment in triplicate in liquid mannite solution as well as in heated soil to which protozoa had been added, and concluded that in liquid media protozoa feed upon azotobacter, checking their activities to a certain extent, while in the heated soil their presence is without any effect. He makes no reference to the fact that even if protozoa consumed azotobacter, it was simply a question of the nitrogen being transferred from the bodies of azotobacter to that of protozoa, since there is no evidence on record to show that protozoa are capable of bringing about denitrification. The utilisation of fluid medium involves so many factors that the results obtained by its use must not be too greatly relied upon: the depth of liquid, the tightness of the plug, the surface of the medium, the action of glass vessels and the question of aeration have all been found to have a tremendous effect on the results. The conditions are not at all comparable to those of soil.

II. EXPERIMENTAL PART.

The investigations recorded here deal with the studies of the influence of protozoa on azotobacter. The writer has made an effort to collect some preliminary data and determine the extent of the action of protozoa on this most important process of free nitrogen-fixation.

Methods.

The protozoon cultures were isolated from different soils and grown with azotobacter and other bacteria on mannite agar of the following composition:

Mannite	20 grms.	Calcium sulphate	0·1 grms.
Calcium carbonate	5 ,,	Mono-potassium phosphate	0·2 ,,
Magnesium sulphate	0·2 ,,	Agar agar	20 ,,
Sodium chloride	0·2 ,,	Water (distilled)	1000 c.c.

(Mono-potassium phosphate was separately dissolved in water and the reaction adjusted to 7·2 *p*H.)

The organisms isolated and made use of are as follows:

Table I.

Showing the detail of organisms used in the experiment.

No. of culture	Organisms present*
1.	Colpoda sp., Hartmanella hyalina, Flagellate sp., Azotobacter and other bacteria.
2.	Colpoda sp., Hartmanella hyalina, Azotobacter and other bacteria.
3.	Heteromita globosus, Azotobacter and other bacteria.
4.	Hartmanella hyalina, Sp. γ†, Azotobacter and other bacteria.
5.	Colpoda sp., Hartmanella hyalina, Ophidomonas sp., Azotobacter and other bacteria.
6.	Hartmanella hyalina, Sp. γ†, Azotobacter and other bacteria.
7.	Hartmanella hyalina, Flagellate sp., Azotobacter and other bacteria.
8.	Hartmanella hyalina, Flagellate sp., Azotobacter and other bacteria.
9.	Colpoda cucullus, Azotobacter and other bacteria.
10.	Azotobacter and other bacteria.
11.	Hartmanella hyalina, Azotobacter and other bacteria.
12.	Azotobacter (pure culture).
13.	Azotobacter (pure culture).
14.	Colpoda sp., Azotobacter and other bacteria.
15.	Colpoda sp., Hartmanella hyalina, Azotobacter and other bacteria.
16.	Colpoda sp., Azotobacter and other bacteria.
17.	Hartmanella hyalina, Flagellate sp., Azotobacter and other bacteria.
18.	Colpidium colpoda (with only one species of bacteria) and Azotobacter.

* I am indebted to Mr H. Sandon of this laboratory for checking the identity of these cultures.

† New species described in the paper. See Reference (3 *a*).

Special care was taken to prepare culture No. 10, *i.e.* control. It contained all the species of bacteria present in the protozoa cultures. The separation of protozoa was carried out by heating a suspension of the mixed culture to 80° C. at which temperature all active forms of protozoa are killed, also by centrifugalising another similar portion and decanting of the upper liquid containing bacteria only. The two were then mixed and utilised as control.

25 c.c. of the media was placed in 250 c.c. Erlenmeyer flasks and after sterilisation allowed to cool in an inclined position so as to obtain slopes, 5 c.c. of sterile water was then added to each flask. (It is important to keep a good supply of moisture over the surface of the medium while the organisms are growing, otherwise the growth will not be uniform.)

Method of inoculation.

(i) In artificial media:

After the stock cultures had grown for about a week, and a thick scum was formed over the surface of the medium—at which time almost all the protozoa are in an encysted condition—three loops were taken for inoculation in each flask.

It is rather important to let the protozoa encyst before transferring them on to fresh medium since there is a large percentage that die if transferred in an active condition, and the inocula will not be uniform.

(ii) Sand cultures:

The method employed for inoculation was that suggested by Mr D. W. Cutler. He found that if protozoa are added to soil or sand in the ordinary way they usually do not grow; one has to be very careful in making inoculation in soil or sand cultures.

The method suggested and employed successfully on previous occasions was to rub in a portion of the scum in the requisite quantity of the fluid media so as to ensure the distribution of the organisms throughout the whole media, and then spray the liquid on to the surface of the sterile sand, so that it penetrated into the entire column of the sand.

Preparation of sand.

A quantity of sand was digested with strong hydrochloric acid for 24 hours and washed free of acid under the water tap and dried. Hundred gramme portions of this were then placed in 250 c.c. Erlenmeyer flasks and sterilised in an autoclave at 130° C. for 30 minutes. The moisture contents were made up by adding the liquid nutrient media used for inoculation.

Examination of cultures while the experiment was in progress.

Artificial culture medium:

Microscopic examination was carried out every fourth day to ensure that the protozoa were growing in the media.

Sand cultures:

A quantity of sand (0·5 grm.) was taken out at an interval of 7th and 14th day, and placed on a quantity of artificial medium in petri dishes with 1 c.c. sterile water, and examined after the growth had taken place.

Direct microscopic examination of a small quantity of sand with a drop of sterile water on the microscopic slide was also made and the protozoa were seen growing in an active condition.

In No. 10 (control) no protozoa developed.

Inoculations in sand were carried out with the help of Mr D. W. Cutler, who also examined cultures before inoculation as well as after the 7th and 15th day.

Incubation temperature and period.

The incubation temperature was the usual room temperature, viz. 20–22° C.

In the first series of experiments the incubation period was only ten days and on testing it was found that a portion of mannite was still present in the cultures, therefore in all the latter experiments the incubation period was 15 days.

Method of nitrogen estimation.

Kjeldahl's method was utilised in determining the nitrogen content of each culture. 20 c.c. of strong sulphuric acid was used for digestion, and after adding the excess of alkali the ammonia was distilled off, receiving the distillate in decinormal sulphuric acid. For titration decinormal sodium hydroxide was used. The indicator employed was tincture cochineal.

I am indebted to Mr H. J. Page, head of the chemical department, for giving me facilities to carry out the estimations in his laboratory.

Accuracy of the experiments.

Each experiment was carried out in duplicate, and in certain cases in triplicate. The results in most cases agreed to a single drop of decinormal sulphuric acid.

Series I. Artificial culture media.

(Incubation period 10 days.)

No. of culture	Organisms* present	Mgms. of nitrogen fixed per gram of mannite	Gain or loss	
			Actual	Per cent.
1	C.A.F.N.†	10·74	+0·14	+ 1·32
5	C.A.F.N.	11·03	+0·43	+ 4·06
2	C.A.N.	10·74	+0·14	+ 1·32
6	A.F.N.	10·88	+0·28	+ 2·64
7	A.F.N.	12·60	+2·00	+18·90
8	A.F.N.	9·74	−0·86	− 8·10
3	F.N.	10·74	+0·14	+ 1·32
11	A.N.	10·45	−0·15	− 1·42
9	C.N.	10·88	+0·28	+ 2·64
10	N. (control)	10·60	—	—

* For detail of organisms please see Table I.

† C. means ciliates; A. amoebae; F. flagellates; and N. nitrogen-fixing bacteria.

Series II. Artificial culture media.

(Incubation period 15 days.)

No. of culture	Organisms* present	Mgms. of nitrogen fixed per gram of mannite		Gain or loss	
		Parallels	Mean	Actual	Per cent.
1	C.A.F.N.†	{11·89 / 12·32}	12·1	+2·07	+20·1
5	C.A.F.N.	{11·6 / 12·17}	11·89	+1·86	+18·06
2	C.A.N.	{11·46 / 12·03}	11·75	+1·72	+16·7
15	C.A.N.	11·6	11·6	+1·57	+15·25
6	A.F.N.	{11·17 / 11·03}	11·1	+1·07	+10·39
4	A.F.N.	{11·46 / 10·88}	11·17	+1·14	+11·07
7	A.F.N.	{12·6 / 12·89}	12·75	+2·72	+26·41
8	A.F.N.	{10·17 / 9·74}	9·96	−0·07	− 0·69
17	A.F.N.	9·74	9·74	−0·29	− 2·81
3	F.N.	{11·17 / 10·88}	11·03	+1·0	+ 9·71
11	A.N.	{10·74 / 10·88}	10·81	+0·78	+ 7·57
9	C.N.	{11·17 / 10·6}	10·89	+0·86	+ 8·35
14	C.N.	12·89	12·89	+2·86	+27·77
10	N. (control)	{10·31 / 9·74}	10·03	—	—

* For detail of organisms please see Table I.

† C. means ciliates; A. amoebae; F. flagellates; and N. nitrogen-fixing bacteria.

Series III. Artificial culture media.

(Incubation period 15 days.)

No. of culture	Organisms* present	Mgms. of nitrogen fixed per gram of mannite: Parallels	Mean	Gain or loss: Actual	Per cent.
5	C.A.F.N.†	10·31, 11·65, 10·17	10·71	+1·12	+11·68
15	C.A.N.	11·03, 10·60	10·82	+1·23	+12·83
7	A.F.N.	12·03, 11·03, 11·17	11·42	+1·83	+19·08
9	C.N.	10·6, 11·11, 10·6	10·77	+1·18	+12·3
14	C.N.	10·54, 10·88	10·71	+1·12	+11·68
10	N (control)	9·59, 9·59	9·59	—	—

* For detail of organisms please see Table I.

† C. means ciliates; A. amoebae; F. flagellates; and N. nitrogen-fixing bacteria.

Series IV. Sand cultures.

(Incubation period 15 days.)

No. of culture	Organisms* present	Mgms. of nitrogen fixed per gram of mannite: Parallels	Mean	Gain or loss: Actual	Per cent.
1	C.A.F.N.†	4·58, 4·58	4·58	−0·58	−11·24
15	C.A.N.	5·73, 6·59	6·16	+1·0	+19·38
6	A.F.N.	5·44, 5·16	5·3	+0·14	+ 2·71
17	A.F.N.	5·73, 4·87	5·3	+0·14	+ 2·71
11	A.N.	6·59, 6·3	6·46	+1·3	+25·19
14	C.N.	6·59, 7·45	7·02	+1·86	+36·04
10	N. (control)	5·16, 5·16	5·16	—	—

* For detail of organisms please see Table I.

† C. means ciliates; A. amoebae; F. flagellates; and N. nitrogen-fixing bacteria.

A special experiment in triplicate was arranged to determine the extent of experimental error, the nitrogen estimations being carried out by three different workers. The results agreed very closely and the biggest difference in individual figures was 0·35 c.c. $N/10$ sulphuric acid, which means only 0·00050120 grm. of nitrogen in a set of duplicate or

triplicate estimations. It may be pointed out here that this difference of 0·35 c.c. of $N/10$ sulphuric acid was experienced only in one case. In majority of tests it ranged between 0 and 0·2 c.c.

Mr R. L. Amoore, of the fermentation laboratory, Mr A. H. Bowden, of the chemical laboratory, and Mr A. N. Puri, of the soil physics department, kindly carried out the estimations for this special experiment, Series III, to whom my best thanks are due.

Series V and VI. Pure cultures of *Colpidium colpoda* and Azotobacter.

(Incubation period 15 days.)

No. of culture	Organisms* present	Mgms. of nitrogen fixed per gram of mannite: Parallels	Mean	Gain or loss: Actual	Per cent.
In artificial culture media:					
18	C.N.†	11·89, 12·46	12·18	+1·22	+11·13
12	N. (control)	11·46, 10·45	10·96	—	—
18	C.N.	11·46, 11·17, 11·31	11·31	+0·91	+ 8·75
12	N. (control)	10·45, 10·45, 10·31	10·4	—	—
In sand cultures:					
18	C.N.	10·02, 9·45, 9·74	9·74	+2·68	+37·96
12	N. (control)	8·02, 6·87, 6·30	7·06	—	—

* For detail of organisms please see Table I.

† C. means pure culture of *Colpidium colpoda* (with only one species of bacteria) and N. means pure culture of Azotobacter.

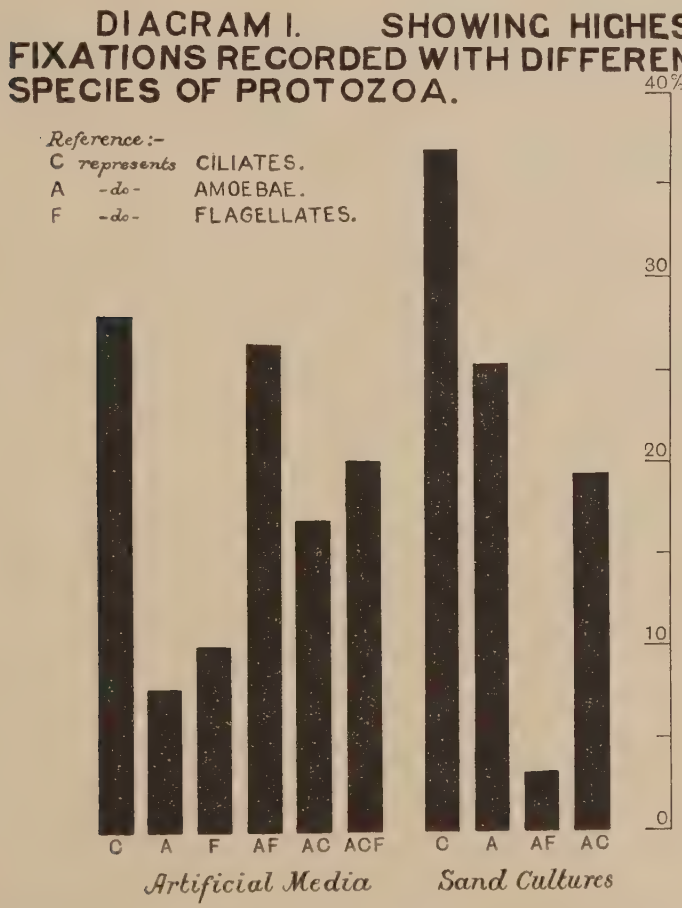
Summary of Results.

A perusal of the above results shows that the presence of protozoa has no depressing effect on the nitrogen-fixing bacteria, either in the artificial culture media, or in sand cultures. From a total of 36 experiments done in duplicates or triplicates, 31 showed a decided gain, while only 5 gave negative results. The average figure for fixation works out to be 8·5 per cent., which is well above the experimental error.

The highest fixation of 36·04 per cent. was recorded in sand cultures in the case of ciliates. All the three types of protozoa gave higher fixation figures. The experiment was repeated six times, and every time concordant results were obtained.

To make the results easily intelligible the highest figures, calculated as percentages, are shown in Diagram I. The vertical columns represent the percentage of nitrogen gained in presence of protozoa after deducting the figures for azotobacter and other bacteria.

At this stage of the experiment it is difficult to say in what manner the protozoa affect the increase of nitrogen-fixation above the value of



azotobacter, whether they themselves take part in actual fixation, independent of the existence of azotobacter, or there is some symbiotic relation between the two types of organisms.

Richards and Sawyer's(11) experiments indicate the importance of protozoa in relation to the nitrogen content of activated sludge. They believe that a large proportion of nitrogen in activated sludge is due

to the bodies of protozoa. In a crude mass culture of protozoa they found as much as 8 per cent. of nitrogen as compared with 7·5 per cent. contained in the richest sample of the activated sludge.

It is intended to extend these experiments so as to test these possibilities.

III.

A final experiment to confirm conclusively the previous results was performed by using a much larger quantity of media, the aim being to obtain bigger differences in the actual fixation values. The highest figure thus far obtained over the control was 2·86 mgms. of nitrogen, and it was thought that probably a large quantity of medium would give a higher value with the same experimental error as before.

The results are shown below:

Series VII. Effect of large quantity of media.

(Incubation period 15 days.)

No. of culture	Organisms* present	Mgms. of nitrogen fixed per gram of mannite	Gain or loss: Actual	Gain or loss: Per cent.
80 *c.c. of media in* 500 *c.c. Erlenmeyer flasks:*				
14	C.N.†	15·75	+2·86	+22·19
7	A.F.N.	14·96	+2·07	+16·06
18	C.N. (pure culture)	13·25	+0·36	+ 2·79
10	N. (control)	12·89	—	—
100 *c.c. of media in large Petri dishes:*				
14	C.N.	24·06	+7·88	+48·7
7	A.F.N.	20·12	+3·94	+24·35
18	C.N. (pure culture)	18·9	+2·72	+16·81
10	N. (control)	16·18	—	—

* For detail of organisms please see Table I.

† C. means ciliates; A. amoebae; F. flagellates; and N. nitrogen-fixing bacteria.

Series VIII. Effect of large quantity of media.

(Incubation period 15 days.)

No. of culture	Organisms* present	Mgms. of nitrogen fixed per gram of mannite: Parallels	Mgms. of nitrogen fixed per gram of mannite: Mean	Gain or loss: Actual	Gain or loss: Per cent.
50 *c.c. of media in large Petri dishes:*					
14	C.N.†	{13·89 / 13·82}	13·86	+3·12	+29·05
7	A.F.N.	{11·96 / 11·89}	11·92	+1·18	+10·99
18	C.N. (pure culture)	{11·38 / 11·60}	11·49	+0·75	+ 6·98
10	N. (control)	{10·74 / 10·81 / 10·67}	10·74	—	—

* For detail of organisms please see Table I.

† C. means ciliates; A. amoebae; F. flagellates; and N. nitrogen-fixing bacteria.

From the above tables it will be seen that large quantities of media have given bigger differences. The highest fixation is 7·88 mgms. in the case of ciliates, which means 48 per cent. net addition of nitrogen over the control experiment.

These figures not only confirm the previous results, but make one feel that the presence of protozoa plays an important part in the fixation of atmospheric nitrogen by azotobacter, and it will be interesting to continue this work with a view to discovering the factor or factors inducing these large fixations.

The work was carried out in Mr D. W. Cutler's laboratory, to whom I have a deep sense of gratitude for his ever-ready help and unfailing kindness. I greatly appreciate his invaluable suggestions and criticisms.

I am indebted to Sir John Russell for his kindness in giving me facilities to carry out the work in his institute, also for his unreserved courtesy, his sympathetic attitude and his keen interest in the work.

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OBSERVATIONS ON THE LIFE-HISTORIES OF *HYPODAERIUM CONOIDEUM* (BLOCH) AND *ECHINOSTOMUM REVOLUTUM* (FROEL): TREMATODE PARASITES OF THE DOMESTIC DUCK

By G. M. VEVERS, M.R.C.S., L.R.C.P., F.Z.S.

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(With 1 Text-figure.)

On May 8th, 1922, a number of encysted Trematodes were found in the liver of a *Limnea peregra* from Hendon, Middlesex. These were found to be larval Holostomes, probably *Tetracotyle typica* Diesing 1858.

This snail was fed to two Ducklings *A* and *B*, two days old, and on the 10th May another snail, also containing similar encysted Holostomes, was divided and fed to each Duckling.

On May 17th a *Limnea Stagnalis* from Italy was dissected and found to be infected with Rediae containing fully developed Echinostome cercariae. These were diagnosed as *Cercaria echinata* Sieb., the larval form of *Echinostomum revolutum* (Froel). The liver of this snail was fed to Duck *A*.

Operculate eggs measuring 100 × 60 were first seen in the faeces on May 28th (20 days after infection). On May 30th Duck *A* died and an examination of the intestine revealed the following parasites:

Fifteen specimens of *Hypodaerium conoideum* (Bloch) from the lower part of the small intestine.

Fifteen specimens of *Echinostomum revolutum* (Froel) from the cloaca.

The specimens of *Hypodaerium conoideum* were mature and the uterus contained fully grown eggs (22 days after infection).

The *Echinostomum revolutum* (12 days after infection) were immature. They measured on an average 4 mm. in length by ·75 mm. in breadth. The oral and ventral suckers were well formed and the head was surrounded by the typical collar of 37 spines. The cuticle of the ventral surface of the body between the head and ventral sucker was thickly

covered with spines which gradually became fewer towards the posterior end of the body. The muscular pharynx and the alimentary caeca were fully developed.

The ovary, shell gland and yolk glands were in process of formation; and the uterus, which contained no eggs, was present as a thin-walled tube running forward from the ovary to the common genital pore, situated immediately in front of the ventral sucker.

The testes, which contained spermatozoa, were ovoid in shape and occupied a position one in front of the other in the posterior half of the body.

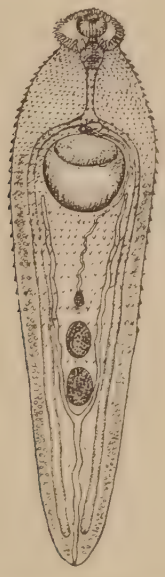

Fig. 1. Immature *Echinostomum revolutum* (Froel) from cloaca of Experimental Duck, 12 days after feeding with *Cercaria echinata* Sieb. (× 20)

The excretory system was Y-shaped, the bifurcation occurring between the posterior testes. Each branch of the Y could be traced forward as far as the ventral sucker. In more mature specimens the excretory system is not so evident. No Holostomes were recovered from Duck *A*.

On June 25th, 48 days after feeding with *Limnea peregra*, Duck *B* was killed; this bird, which had been passing fluke eggs since May 28th, was very thin and weak.

Forty specimens of *Hypodaerium conoideum* were recovered from low down in the small intestine. There was a localised inflammation of the mucous lining of the intestine over the area occupied by the flukes.

Although many encysted Tetracotyle were ingested by both ducks, no Holostomes were recovered from either. The specimens of Hypo-

daerium which were recovered from both ducks must have developed from some other cercariae also encysted in *Limnea peregra*, but not detected at the time of the examination of the snails.

Conclusions.

(1) That the intermediate host of *Hypodaerium conoideum* (Bloch) is *Limnea peregra*, the larval form being a cercaria, as yet undetermined, which encysts in some organ of that snail.

(2) That these flukes reach maturity in the small intestine of the duck in 20 days.

(3) That when present in large numbers they cause a considerable inflammatory reaction in the small intestine and may be responsible for a certain amount of debility and wasting.

(4) That *Echinostomum revolutum* (Froel) is Protandrous, the male sexual organs in the young form being in a more advanced stage of development than the female.

(5) That the encysted larval Holostome (*Tetracotyle typica* Diesing) from *Limnea peregra* does not develop in the domestic duck, or if it does, it produces adults so small as to be easily passed over at a post-mortem examination.

(*Received November 11th*, 1922.)

THE ACTION OF PROTOZOA ON BACTERIA WHEN INOCULATED INTO STERILE SOIL

By D. WARD CUTLER, M.A.

(*From the Protozoology Department, Rothamsted.*)

(With 1 Text-figure.)

It has been shown that in normal field soil the bacteria and active amoebae show an inverse relationship when their numbers are counted on successive days, and the conclusion has been drawn that the protozoa are in part responsible for inhibiting the normal increase in bacterial numbers[1]; but the further proof of this action, which ought to be obtained by inoculation experiments, has never been given.

Both in this country and abroad such proof has been sought, but with unsatisfactory results since in many cases there is no published evidence that the protozoa inoculated into soil survived in their new environment; while in others the countings were made at such long intervals as to preclude the possibility of deduction. Goodey[2], however, does record a case in which the bacterial numbers were reduced after the inoculation of amoebae cysts. He says, however, that there is no proof that excystation ever occurred and therefore does not connect the drop in numbers with the presence of protozoa.

In view then of the admitted crucial nature of such a test the following experiment was performed.

Ordinary field soil from a plot of ground annually manured with 14 tons of farmyard manure was sterilized in the steamer for one hour on four successive days. Tests showed that this had rendered the soil sterile. It was then divided into three portions each of 100 grms., which were placed in large sterile Petri dishes ready for inoculation. The inocula used were

(*a*) Bacteria alone.

(*b*) „ + one species of amoebae (*Dimastigamoeba gruberi*).

(*c*) „ + one species of flagellate (*Cercomonas crassicauda*).

[1] *Phil. Trans. Roy. Soc.* B, vol. 211, pp. 317–350.

[2] *Proc. Roy. Soc.* B, vol. 89, pp. 297–314.

These were prepared as follows. Each of the species of protozoa had been so purified of bacteria that only three species remained, each of them being common soil bacilli. These were isolated free from protozoa and a suspension in sterile physiological saline prepared and counted in a counting apparatus. The two inocula of protozoa + bacteria were then made in similar suspensions so that each contained the same number of bacteria as did the control (protozoa free) suspension. In each case also most of the protozoa were in the encysted condition. This was important for previous experiments had shown that inoculations with active forms were rarely successful, the organisms usually dying soon after reaching the soil. Further it was found that inoculation by the addition of a given quantity of fluid to one portion of the soil was unsatisfactory, since often death of the active forms occurred; and also it took a considerable time for the protozoa and bacteria to spread uniformly through the soil sample.

In the present experiment therefore the inoculations were done by the use of a throat spray fitted with the finest nozzle obtainable[1].

By this means, however, it is difficult to record directly the size of the inoculations; so to ensure that approximately the same numbers of bacteria were added to each soil, the dishes and their contents were first weighed and then spraying the inocula was continued, until the same quantity by weight of fluid had been given, and the H_2O content of each soil had been brought to approximately 15 per cent.[2] Throughout the spraying the soils were shaken to ensure as far as possible uniform distribution.

Immediately after the completion of these operations counts by the plate method were made of both protozoa and bacteria; as will be seen by reference to the table the numbers of bacteria added to each soil were practically the same. The total numbers of protozoa added were:

Dimastigamoeba gruberi: 25,000 per gram.
Cercomonas crassicauda: 20,000 „

The soils were then incubated at a temperature of 20° C. For the first eight days after inoculation the numbers of bacteria were counted daily by taking from each dish 5 grms. of soil which was diluted to 1/1,000,000 in sterile physiological saline and plated out in the usual way. It was realised that 5 grms. was a small portion of soil to use, but this was the

[1] It may be mentioned here that this method has proved most satisfactory, and was used in the experiments detailed in a paper by Mr Nasir and published on p. 122 of this Journal.

[2] Every other day the soils were weighed and the water content brought to the 15 per cent. level by the addition of sterile tap water.

largest quantity practicable if the counts were to be long continued: moreover previous experiments had demonstrated that reasonably accurate comparative counts could be so made.

The protozoa for this first period were not counted. At the end of eight days counting was discontinued for a week, and then commenced once more for seven days. For this period the complete enumeration of the active and cystic protozoa was not done, but certain tests were made which demonstrated that these organisms were active in the soil, and on the fifteenth day the total numbers of protozoa were: *D. gruberi* 230,000 per gram; *C. crassicauda* 420,000 per gram.

The control soil throughout the experiment remained uncontaminated with protozoa.

In Table I and Fig. 1 the results of the experiment are set out.

Table I.

The numbers of bacteria are given in millions per gramme of soil.

Number of days after inoculation	Control (bacteria alone)	Control bacteria + amoebae	Control bacteria + flagellates
0	13·0	12·2	11·4
1	48·6	35·4	26·4
2	97·6	117·2	65·6
3	127·0	178·4	96·8
4	154·8	154·4	68·8
5	196·8	177·0	93·4
6	214·4	151·8	95·4
7	193·4	75·6	103·0
8	165·2	65·8	90·4
15	169·2	72·8	88·0
16	174·8	30·2	51·6
17	175·6	53·2	59·2
18	168·4	82·8	41·2
19	160·4	43·8	68·2
20	171·2	70·8	43·0
21	176·2	28·2	76·6

It will be noted that the numbers of bacteria in each of the soils rise steadily until they reach a maximum 6–8 days after the inoculations were made. This agrees with expectation since the bacteria multiply much more rapidly than do protozoa, which until their active forms are numerous are unable to exert any appreciable influence on the bacterial population. Also it will be remembered that the protozoa were inoculated as cysts, which, judging from analogy in fluid cultures, will probably remain as such for relatively long periods (24 28 hrs.) before excystation occurs.

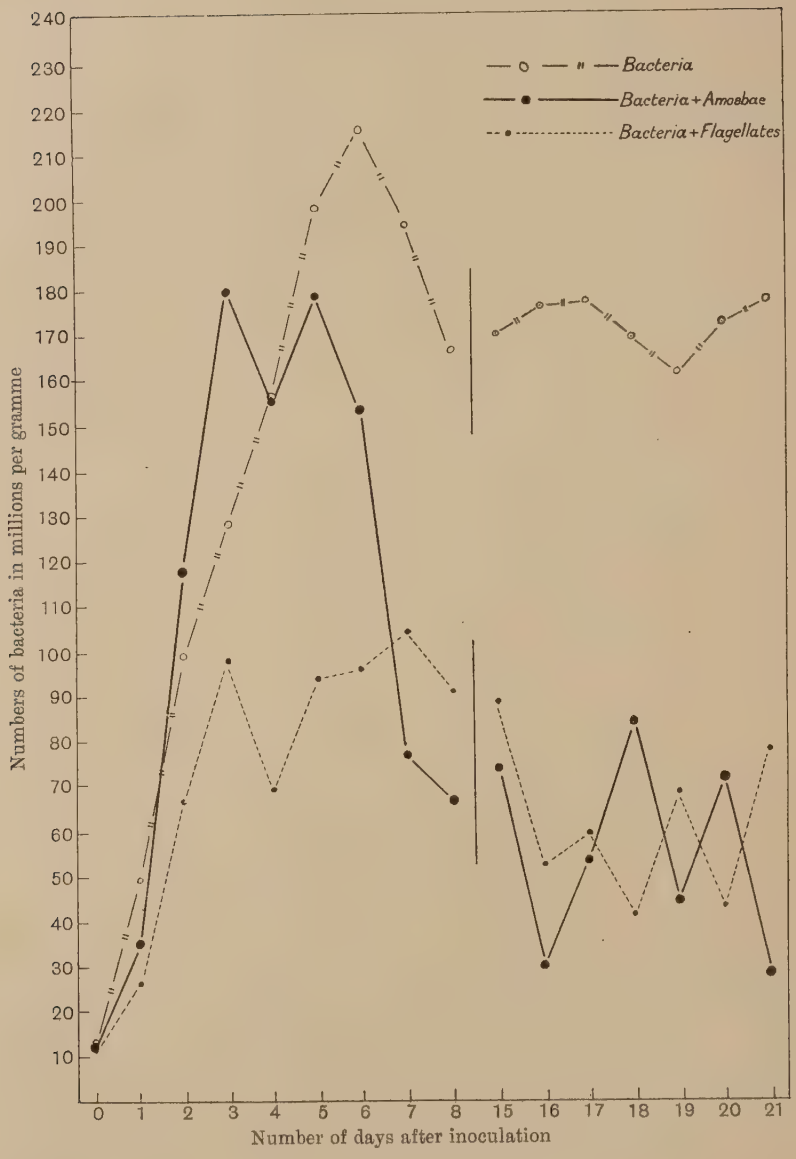
Bacteria
Bacteria + Amoebae
Bacteria + Flagellates
Numbers of bacteria in millions per gramme
Number of days after inoculation
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 1 2 3 4 5 6 7 8 15 16 17 18 19 20 21

Fig. 1.

In the case of the control and the bacteria + amoebae soils the final numbers attained are almost the same, 214·4 and 177·0 respectively; for some inexplicable reason, however, in the bacteria + flagellate soil the numbers of bacteria did not rise above 103·0 millions per gram.

The most interesting part of the experiment however, is the comparison of the bacterial numbers in the three sets of soils for the last seven counts, that is 15–21 days after inoculation. During this period the protozoa were known to be leading an active existence in the soil, and the relationship normally existing between them and the bacteria appears to have been attained.

In the soil containing only bacteria (control) the numbers for the last seven days of the experiment remained constant: the largest difference in two counts being only 11·2 millions per gram, which is not significant on the assumption that the error was 5 per cent.

In the other soils, on the other hand, the variations in the number of bacteria are considerable, especially as regards the soil containing amoebae, and are well outside the experimental error. Indeed the variations in numbers are comparable with those found from day to day in ordinary field soils under normal conditions and treatment. The soil containing flagellates (Cercomonas) does not show the fluctuations to the same extent, but again this is in accord with expectation, since Cercomonas, though an organism known to feed on bacteria, is not so voracious as *Dimastigamoeba gruberi*, the organism living in the soil containing amoebae.

Finally the experiment shows in a marked way that the bacteria in the soil free from protozoa are able to maintain their high numbers for a longer period than those living in soils containing protozoa.

In conclusion therefore it may be claimed that this experiment amply confirms the conclusion arrived that from other data, viz. that in normal field soil the bacteria and active amoebae show an inverse relationship: and that in such soils the presence of active protozoa are one of the factors concerned in keeping the numbers of bacteria below the level they might have otherwise attained.

(*Received November 16th*, 1922.)

STUDIES IN THE VARIETAL IMMUNITY OF POTATOES TO WART DISEASE (*SYNCHYTRIUM ENDOBIOTICUM* SCHILB., PERC.)

PART I

THE INFLUENCE OF THE FOLIAGE ON THE TUBER AS SHOWN BY GRAFTING

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Introduction.

This paper is an account of a first attempt to answer one of the many questions which arise when a biochemical study of the nature of varietal immunity to disease in plants is undertaken. The investigation is a section of the larger study of the problems of immunity to Wart Disease of potatoes and the control of this disease, in which the Department of Mycology and the Insecticides and Fungicides Department are collaborating.

It is well known that there are varieties of potatoes which have never been known to take Wart Disease; and the opinion has been held by pathologists for some time that this immunity is absolute, and not merely relative, as is the case in most plant diseases. Breeding experiments, notably those of Salaman and Lesley (1), tend to confirm this opinion. This apparently clear-cut distinction between "immunes" and "susceptibles" makes the case of Wart Disease of potatoes a good one for the biochemical study of the nature of varietal immunity to disease.

There is singularly little knowledge however of the general nature of this immunity. It may for example be conferred by a definite substance not present in the susceptible varieties, or on the other hand it may be due to the absence in the immune varieties of a substance which makes growth of *Synchytrium endobioticum* possible. If either of these suppositions be correct, this substance may be produced throughout the whole plant or it may be produced in one part and translocated thence to the remaining tissues. The possibility that the difference between "immunes" and "susceptibles" is the result of slightly different chemical

groupings in the protoplasm in the two cases, does not seem to have been explored. Information of this nature is highly desirable before a biochemical study of the problem is undertaken; for each of the numerous possibilities, of which only a few are mentioned above, necessitates a different mode of attack.

Consideration of the functions of the various organs of the plant does not make one hypothesis more or less likely to be correct than another, nor does it help in the selection of any particular organ of the plant which is more likely to repay investigation than another. Mechanisms may be present whereby the root, the foliage and the stolon-tuber elements may be responsible either singly or in conjunction with each other.

It was thought that light might be thrown on such questions and guidance obtained in the selection of the portion of the plant to be investigated first by exchanging elements of immune plants for corresponding ones from susceptible plants by means of grafting, and observing the effect on the tubers.

This paper deals only with the question whether the immunity or susceptibility of the tubers is in any way affected by the foliage which is responsible, in part at least, for synthetising the chemical compounds of which they are composed. The effect of the root, stolon and tuber part on the immunity or the susceptibility of the foliage, and the separate functions of the root, stolon and tuber elements are under investigation.

Experimental.

A healthy rapid growth of the plant was found to be an almost indispensable condition to success in grafting. The potatoes were grown in unglazed earthenware pots (11′ diameter) in a greenhouse, and a depth of 1″ to 2″ of sand was placed in the bottom of the pot to prevent water-logging of the soil. The pot was filled to within 1″ of the top with a mixture of equal parts of sand, finely sifted clay soil and finely sifted well decomposed farmyard manure; and the remaining inch filled with clean sand. The purpose of the latter was to prevent "caking" of the soil surface and excessive loss of moisture and to keep the potato stem clean. The pots were stood in saucers, and watering was only done when the leaves showed signs of withering in the hottest part of the day. The water was given in the saucers and the soil allowed to take it up slowly, thus avoiding the risk of water-logging.

Grafting was commenced when the plants were about 3″ to 4″ high, and the larger leaves were cut off to prevent wilting due to excessive evaporation from the scion. Plants were grafted usually in pairs that

the "heads" might be simply exchanged; in this way both time and material were economised. After observing that the stems were clean, they were cut about 1″ above the sand, and the detached "heads" interchanged, care being taken to keep the cut surfaces clean. In each case the stem of the scion was sharpened into a wedge by two clean cuts, a slit made in the stem of the stock, and the wedge introduced so as to make a tight junction along the whole cut surfaces[1]. Success in grafting seemed to depend very largely on the speed and neatness with which these two last operations were carried out. The scion was tied in with artificial silk as tightly as possible without damaging the tissues unduly, and the joint was made air-tight by painting with paraffin wax kept molten on a steam bath. The whole plant was removed from the soil and all tubers larger than a sweet pea cut off; it was replaced in the pot, watered well from the top and placed in the shade, where it was allowed to remain until the scion was growing strongly. By observing these precautions it was found possible in most cases to prevent any appreciable loss of turgor in the scion, and when union was well established the paraffin wax and binding were removed.

Few plants were lost through infection at the graft; and this method was found to give better results than any in which the plants were disinfected. The tubers resulting from these grafts varied in size up to about 1½″ diameter. These were grown in infected soil, with the results shown below. In the table on p. 145 the composite plants are arranged in types according to whether the scion and stock are immune or susceptible.

It will be noticed that all tubers from types "immune on immune" and "susceptible on immune" give progeny free from Wart Disease, while all from types "immune on susceptible" and "susceptible on susceptible" give progeny which are infected. So thorough was the infection in the last two types that no tuber larger than 4 × 3 × 2 cms. escaped the disease and only a few smaller than this size were found clean. In most cases every eye of the tuber was infected.

Though the number of experiments was small these clear-cut results seem to justify the conclusion that in these experiments the immunity or susceptibility of the tubers arising from the stock is unaffected by the scion. The grafting of "susceptible" foliage on to an "immune" underground portion has not resulted in infection of a single "eye." There is no evidence of decreased infection in "immune on susceptible" type as compared with "susceptible on susceptible" type, as might be expected

[1] A piece of "Gillette blade" soldered into a metal handle was found a very convenient grafting implement both because of its sharpness and its thinness, which avoided bruising of the cut tissues.

Results of susceptibility tests on tubers obtained from "composite plants."

Graft no.	Foliage / Below ground portion	Types	No. of Tubers obtained from composite plant	"Set" no.	No. of Tubers: Clean	No. of Tubers: Infected	No. of "Eyes": Clean	No. of "Eyes": Infected	
45	Arran Comrade / Tinwald Perfection	Immune / Immune	1		2	0	10	0	Clean
53	Kerr's Pink / Arran Comrade	Immune / Immune	2	(1) / (2)	4 / 3	0 / 0	16 / 10	0 / 0	Clean
54	Kerr's Pink / Ally	Immune / Immune	1		5	0	18	0	Clean
37	Arran Chief / Ally	Susceptible / Immune	1	(1) / (2)	3 / 2	0 / 0	16 / 9	0 / 0	Clean
40	King Edward / Ally	Susceptible / Immune	2		8	0	40	0	Clean
115	Up to Date / Dargill Early	Susceptible / Immune	2		3	0	?	0	Clean
30†	Great Scot / King Edward	Immune / Susceptible	1		0	3	8	12	Warted
39	Ally / King Edward	Immune / Susceptible	1		4	7	17	23	Warted
50	Arran Comrade / Arran Chief	Immune / Susceptible	1		0	7	0	21	Warted
57	Kerr's Pink / Arran Chief	Immune / Susceptible	2		0	6	1	13*	Warted
41	King Edward / Arran Chief	Susceptible / Susceptible	1		0	8	4	19	Warted
135*	Arran Chief / Alnwick Castle	Susceptible / Susceptible	1		0	1	0	1*	Warted

* Large masses of wart.

† In this experiment the old tuber was not removed. Seeing that a considerable amount of synthesis was to be expected from the quantity of foliage developed and that the young tuber seemed the only place where the products could be stored, the experiment was thought worth including.

to be the case if the "immune" scion had any influence on the "susceptible" stock.

Before leaving this work it is hoped to produce a few larger crops of tubers from grafted plants from which, on grafting, all stolons as well as tubers were removed; to increase the number of experiments; and to grow the actual grafted plant in infected soil and not the produce alone as in the above piece of work.

These results suggest that if immunity or susceptibility be due to a substance produced in one part of the plant and translocated thence to the other parts, then the foliage is not the source of this substance. Experiments are in hand to determine whether the below-ground portion has any such effect on the foliage, and these should indicate whether such a substance is produced in the former part. If it is, the effects of the separate units of the below-ground portion may be analysed further,

whilst if there is no such effect, the hypothesis of some structural chemical difference between the products of the protoplasmic activities of "immunes" and "susceptibles" would seem worth testing.

Because of the smallness of the amount of data, this work is to be regarded as preliminary, but the fact that all the results are susceptible to one interpretation only seemed to warrant their publication.

It is a pleasure to express my indebtedness to Mr F. Tattersfield and Dr W. B. Brierley for much helpful criticism.

Summary.

1. Grafting experiments of a preliminary nature have been carried out to throw light on the functions of the various organs of the potato plant in rendering the tubers immune or susceptible to Wart Disease (*Synchytrium endobioticum* Schilb., Perc.).

2. It is hoped that such knowledge may facilitate a biochemical study of the problem by indicating which part of the plant to investigate first, and to give some clue to the general nature of the chemical differences to be sought.

3. Composite plants were built up by grafting in the following ways:

3	plants of the type	Immune	grafted on	Immune.	
3	,,	,,	Susceptible	,,	,,
4	,,	,,	Immune	,,	Susceptible.
2	,,	,,	Susceptible	,,	,,

4. The results are in harmony with the conclusion that the character of the foliage has no influence on the immunity or the susceptibility of the tubers to Wart Disease.

5. It follows that no compound synthetised in the leaves is likely to be responsible for separating potatoes into "immunes" and "susceptibles."

6. The investigation is being continued, and it is hoped to determine whether any one part of the plant influences the immunity or susceptibility to the disease of any other part. The desirability of investigating a portion possessing such an influence is obvious. If the several parts of the plant have no such effect on each other, a search for differences in substances, such as the proteins, which are only translocated with difficulty, if at all, would be indicated.

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(*Received January 26th*, 1923.)

REVIEW

The Coccidae of Ceylon. By Green, E. E. London. Dulau & Co. 1922. Part V, pp. 345–472. Plates cxxxiii–cxci *a*. £3.

The issue of the final part of *The Coccidae of Ceylon* marks the completion of the most important of the many works that have appeared on scale insects. The first part of Mr Green's treatise was published in 1896 and the others appeared successively in 1899, 1904, 1909 and 1922. The volume under review, like its predecessors, represents a very large amount of detailed labour requiring close application with the microscope. Its value is greatly enhanced by the profusion of plates illustrating almost every important structural point in the species dealt with. The excellence of these plates will commend itself to general admiration but, good as these illustrations are, they are excelled by the author's original drawings. It is a matter for regret that few, if any, methods of illustration are capable of reproducing natural objects with the identical fidelity and delicacy of the human hand.

The island of Ceylon represents a limited area, nevertheless its fauna is one of tropical richness, and nearly 300 species of Coccidae have been recorded from within its limits. A number of the species, it is true, are cosmopolitan or nearly so, but the total Coccid fauna of Ceylon includes a very considerable percentage of that of the world. A work of this kind is particularly valuable to Indian entomologists, not only in view of the great economic importance of the family concerned, but also from the fact that the Indian Coccidae are much less known and have not, so far, been comprehensively dealt with.

It is inevitable in a monograph whose publication has extended over 26 years that radical nomenclatorial and other changes have resulted in the meantime: these fortunately are dealt with in an appendix at the end of this volume. We can sympathise with the author in his reluctance to discard such familiar names as *Coccus* for the cochineal scales and *Lecanium* for the brown scales in conformity with the law of priority. These and other changes are particularly confusing to the non-specialist since, in several cases, the discarded names have still to be retained although in connection with totally different genera.

Among features of the present volume is the occurrence of only a single species (*Kuwania zeylanica*) of Margarodinae in Ceylon. Three species of *Margarodes* are found in S. India, but none so far have been detected in the island fauna. Similarly, *Tachardia lacca*, which has a wide range in India and Burma, is not indigenous to Ceylon. This discontinuity in certain elements of faunae of the two regions is well known to be borne out among the larger animals also. Among insects it is perhaps not always a real one, and the discovery of forms such as *Margarodes* in Ceylon is within the bounds of probability.

In conclusion, it may be said that all entomologists will congratulate Mr Green upon the completion of his laborious task. The uniform excellence of the plates, which number 209 in the whole work, is in itself a great achievement. It may also be added that the manner in which these plates are reproduced is no mean tribute to Dutch lithography.

A. D. I.

REPORT OF THE COUNCIL

OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS FOR THE YEAR 1922–3. PRESENTED TO THE ANNUAL GENERAL MEETING, JANUARY 26TH, 1923.

DURING the year seven meetings have been held which have usually been devoted to discussions. The meetings have been well attended, an average of 64 members and guests being present.

A Field Meeting was held during the summer when the Association met at the Royal Horticultural Society's garden, Wisley, at the invitation of the Director, Mr F. J. Chittenden, and the work in progress was demonstrated by the Director and his colleagues.

Reversion to a previous custom was made in the holding of a provincial meeting at Manchester on Dec. 15th and 16th, the local arrangements being carried out by Dr Wilfrid Robinson. The afternoon and early evening were devoted to communications; members were entertained to tea by Professor F. E. Weiss and his colleagues, and an Association Dinner was held in the evening. On the second day the Shirley Institute of the British Cotton Industry Research Association was visited, members being welcomed by the Director, Dr Crossley, and having the work in progress demonstrated to them by the several heads of departments.

During the year the Association sustained a great loss by the death of Mr Bacot, a member of your Council, and an obituary notice was printed in the *Annals of Applied Biology*. Professor J. H. Priestley was invited to fill the vacancy. Since the last Annual General Meeting eleven candidates have been admitted to ordinary membership of the Association and the number of members exclusive of those whose subscription is three or more years in arrear now stands at 234, a decrease of 6 as compared with last year.

Your Council considers that the scientific progress of the Association has been well maintained during the year, but wishes to urge the desirability of introducing as members of the Association additional active workers who may assist in its development.

The thanks of the Association are due to Professor J. B. Farmer and his colleagues for granting the use of rooms for the meetings of your Council, and, further, for their unfailing kindness and hospitality in permitting the Association to meet in the Botanical Lecture Theatre of the Imperial College of Science.